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SEPTEMBER, 1931

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Official Organ of
The American Phytopathological Society

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PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

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PHYTOPATHOLOGY

VOLUME 21 NUMBER 9

SEPTEMBER, 1931

THE ANTIBIOSIS OF CERTAIN BACTERIA TO SMUTS AND SOME OTHER FUNGI[1]

DELIA E. JOHNSON[2]

INTRODUCTION

In the course of some investigations on corn smut in 1926 and 1927, Bamberg (1) found that gall production often failed to occur upon susceptible plants when these were inoculated with the proper combinations of corn-smut lines of known virulence. In a large percentage of cases only a discolored area was produced at the point of inoculation. From such discolored areas bacteria were frequently isolated that were distinctly antibiotic to several species of smuts.

Bamberg found that the bacteria destroyed colonies of *Ustilago zeae* (Beckm.) Ung. of 9 days' growth when he transferred a small amount of the bacterial culture to the edge of smut colonies, the latter being reduced to a slimy mass with only a few sporidia present. In hanging-drop preparations of both the sporidia and the bacteria the former were destroyed in a few days. He also found that the bacteria checked further development of the smut galls on corn plants when he inoculated a suspension of them into the galls with a hypodermic syringe. Inoculation with corn-smut cultures in combination with the bacterial culture usually did not result in gall formation in corn plants. The bacteria also destroyed colonies of *U. zeae*, *U. avenae* (Pers.) Jens., *U. levis* (K. & S.) Mag., *Tilletia tritici* (Bjerk.) Wint., and *Sorosporium reilianum* (Kühn) McAlp. This bacterial culture was given to the writer to determine, if possible, what factors might be responsible for the phenomenon. The writer had also isolated three cultures of bacteria that were antibiotic to several smuts and certain other fungi. This paper deals with the investigations on these cultures. Bamberg called his culture B–1, and the same designation will be used in this paper. The other three cultures are referred to as C–1, D, and *Myxobacterium*–1.

[1] Paper No. 1002 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer wishes to express her indebtedness to Dr. E. C. Stakman, who suggested the investigation and offered valuable suggestions. She also acknowledges helpful criticism from Dr. Leach and many favors from M. B. Moore, J. M. Walter, and R. H. Bamberg of the Plant Pathology Department.

EXPERIMENTAL WORK ON BACTERIAL CULTURE B–1

Bamberg's culture consisted of a coccus and a rod-like bacterium. He was not certain whether he had a pleomorphic organism or a mixed culture, which could not be purified by repeated plating on account of the viscosity of the growth. The writer also was unable to separate the two bacteria by plating. However, the rod-like form had a higher thermal death point than the coccus and was obtained in pure culture by heating the mixed culture slightly above the thermal death point of the coccus. In the course of the filtration experiments mentioned later, a coarse filter was used through which the coccus, but not the rod-like form, passed. In this way a pure culture of the coccus was obtained. It was soon found that the coccus was the antibiotic organism and the rod-like form had no effect upon sporidial growth. The designation B–1 is therefore retained for the coccus culture in this discussion.

The writer repeated some of Bamberg's experiments and found that the sporidia were destroyed in about two weeks in large colonies of *Ustilago zeae, U. avenae, U. levis,* and *Sorosporium reilianum,* when these were inoculated at the edge with the bacterial culture. The sporidia of the smut colony were gradually replaced by a slimy mass of bacteria. The same result was obtained when a small amount of the bacterial culture was placed on top of large colonies of the smut. The sporidia were destroyed also in hanging drops prepared from bacterial and sporidial suspensions in tap water, the bacteria increasing greatly in number, while globules and débris were observed in increasing quantities in the preparation. When the bacterium was inoculated into corn plants in combination with the proper sporidial lines of smut, the plants often failed to develop galls, although galls were present in the checks. When it was inoculated with a hypodermic syringe into galls, these frequently shriveled up, while galls inoculated with sterile water were not so affected. The effect of the bacteria was more marked on young galls than on older ones, the membrane of the former often turning green, all traces of the gall being obliterated as the growth of the plants progressed. The mature galls generally dried up, but the host tissue had been replaced by the fungus tissue to such an extent that chlorophyll production did not occur. The inoculation with sterile water and with a water suspension of the bacterium without sporidia had no effect upon corn plants. In another series of experiments the bacterium seemed to have very little effect upon the various smuts.

These variations in the antibiotic effect of the bacterium seemed rather puzzling. However, two explanations suggested themselves. One might be loss of virulence under certain conditions. The bacterial cultures used, especially in the second series, had been cultivated for some time upon artificial media, and this might have affected the virulence. Checks were made

to determine this, and the results showed that when the bacterium had been cultivated for several months upon artificial media, it was no longer able to attack the smut cultures nor to inhibit gall formation in corn plants. In some of the stock cultures the virulence could not be revived, but in others it was "stepped up" by successive inoculations simultaneously with small amounts of smut growth into media suitable for the growth of both. When such bacterial cultures were combined with the proper sporidial lines and inoculated into corn plants, many of the plants showed no infection, although galls formed in the checks. Another explanation of failure of the bacteria to inhibit gall formation in corn plants might be that the sporidia were present in preponderating numbers in the inoculum and that the bacteria could not multiply fast enough to prevent infection. It is obvious that the survival of only two sporidia of proper affinities may result in the infection of a plant. To check on this possibility, varying amounts of the bacterium were combined with constant amounts of the sporidia and inoculations were made from these combinations into corn plants. Inhibition of gall formation occurred constantly when a highly concentrated suspension of an active culture of the bacterium was used. The failure of the bacterium in the earlier experiments to show consistently properties antibiotic to smuts was probably due to a loss of virulence from prolonged cultivation upon artificial media as well as to a perponderance of viable smut sporidia in the inoculum. Failure of the bacterium to prevent gall formation in the corn plants does not necessarily indicate a lack of antibiotic ability under proper conditions.

The bacterium seems to have no effect on old smut cultures. At first this was thought due to the dryness of the smut culture, but the addition of sterile water at the time of inoculation with the bacterium did not increase its activity.

As was found by Bamberg, the organism is most active at fairly high temperatures, 20° to 25° C., although it grows at 10° C. and retains its antibiotic property. No attempt was made to grow it at lower temperatures.

Very little is known about the way in which bacteria may attack fungi. It is evident that the action upon the fungus is either extracellular entirely or extracellular at first and intracellular later. Tolaas (22) and Paine (14) found a fluorescent bacterium that produces disfiguring spots upon cultivated mushrooms. But neither of these two investigators made any study of the mode of attack of this bacterium. Petri (15) of Portici, Italy, in 1927, described a bacterium that penetrates and destroys the hyphae of Phytophthora and Pythiacystis species. Similarly, Sanzone (17) of the same station reported a bacterium which lives symbiotically within the hyphae and conidia of *Fusarium solani* (Mart.) Sacc. Geitler (7) found a bacterium, parasitic upon Cladophora, which destroys the alga by pene-

trating the cells. However, neither Bamberg nor the writer has been able to find any indication that bacterium B–1 enters the hyphae or the sporidia of the smut. This suggestes that the active principle is extracellular and capable of breaking down the cell wall. Such a substance might be an enzyme. Therefore, a study of the enzymes of the bacterium and an analysis of the chemical constituents of the cell wall of the sporidia seemed necessary.

Considerable work has been done on the composition of the cell wall of the lower Thallophytes. A recent work on the subject is by Wettstein (24), who also summarizes the work of other investigators in this field. According to their findings, the Zygomycetes, one of the subgroups of the Phycomycetes, contain chitin in the cell wall; and the Oomycetes, the other subgroup, contain cellulose. In the Ascomycetes chitin has been found to be the principal constituent, but other substances are often present. Wettstein makes the following statement in regard to the Basidiomycetes: "Die Basidiomyceten sind, was das Vorkommen von Chitin betrifft, gleichfalls einheitlich. Es ist auch hier die überwiegende Membransubstanz in fast allen Gruppen. Bei manchen treten wieder andere Substanzen hinzu, die neben Chitin vorhanden sind, oder auch dieses ersetzen können. Cellulose fehlt vollständig. Bei abgeleiteteren Gruppen, Polyporeen, Gasteromyceten, scheint die Zahl der verschiedenen Membransubstanzen sehr gross zu sein." Wettstein makes no mention of smuts and rusts. It is probable that no tests were made on these groups. Microchemical tests for the presence of chitin and pectin in the cell wall of corn smut were therefore made by the writer.

CHEMICAL COMPOSITION OF THE CELL WALL OF CORN-SMUT SPORIDIA

Chitin test. The test for chitin is made by boiling the material in concentrated KOH for about 30 minutes to change the chitin to chitosan. It is then washed in 95 per cent alcohol to harden, and a solution of IKI, the reagent for chitosan is added. A red violet color indicates the presence of chitosan.

When so treated portions of individual sporidia stain red while the remainder of the sporidium is colorless. The red violet areas indicate the presence of chitin; and the colorless areas some other chemical constituent. The stained sporidial mass is red, possibly indicating a preponderance of chitin.

Pectin tests. A. Ruthenium red. A small amount of sporidial material was placed on a slide and a dilute solution of Ruthenium red (1–10,000) was added, the slide being kept in the dark. After about 20 minutes the reagent was washed off. A red color appeared in the sporidial mass and in patches in the individual sporidia. This reaction indicates the presence of pectin in scattered areas of the cell wall.

B. Methylene blue. Pectic substances stain violet with methylene blue (1–1,000), and cellulose usually stains blue. When methylene blue was added to the sporidial material on the slide, a violet color occurred in patches on the walls of the sporidia, other areas being blue. The violet reaction is more marked in young sporidia; and the blue, in the old. This result suggests the presence of pectin and possibly other materials such as cellulose or related substances, in the cell wall of the sporidia. These findings are somewhat in accord with Wettstein's statement that in some groups the number of different membrane substances seems to be very large.

TESTS FOR ENZYMES IN BACTERIUM B–1

Filtration experiments. Bamberg found that the addition of a small amount of the filtrate of a broth culture of B–1 had no effect upon corn smut. The writer filtered both fresh and old broth cultures of the bacterium with a fine filter, which eliminated the bacterium. Smut always grew readily in it. This was repeated many times with the same results. It indicates that there is no exhaustion of the food supply by the bacterium which might be responsible for the destruction of the smut. It also shows that there is not a sufficient amount of enzymes or toxins present in the filtrate to affect the smut. However, Jones (9), in working upon enzymes of *Bacillus carotovorus* found that a large portion of the enzyme is adsorbed by the filter. He also found that a direct extraction of the enzyme with the proper reagents gives a much larger amount of it. Among such reagents, he used alcohol in sufficient quantities to make the broth culture 80 per cent alcoholic, then filtered it, and dried it quickly. This method was used in the present investigation of enzymes produced by this bacterium. Broth cultures of the organism were centrifuged, the bacteria removed with a small amount of the broth, shaken with sterilized glass beads until most of the bacteria were crushed, and finally extracted with alcohol, and filtered. Checks were run on the technique by making similar extractions from *B. carotovorus*. The residues from both cultures were tested for pectinase on slices of surface-sterilized carrot. Both broke down the cells of the carrot, reducing it to a soft mass resembling that of soft rot. The action of the residue of the crushed bacteria of Bamberg's culture was several times as rapid as that of the residue from the broth. Contamination was guarded against by the use of 1 per cent toluol. Inoculations were made from the carrot slices into broth to determine if any contamination might have occurred which could be responsible for the breaking down of carrot tissue. Only a spore-bearing bacterium was found, and this had no effect upon carrot. This also showed that Bamberg's culture had been killed by the alcohol and could not have produced the softening of the tissue. A further test on this point was made by inoculation of carrot slices with the or-

ganism, but it had no effect on them. Apparently, there is something present in the alcoholic extract of the bacterium which breaks down pectic material, but the organism itself is unable to attack carrot and produce soft rot in it.

It was difficult to obtain any definite and conclusive results in regard to the effect of the same type of residue on smuts in hanging-drop preparations. Jones states (9) that the residue of *Bacillus carotovorus* consists of precipitated proteins, bacterial cells, and the precipitated enzymes. That is probably true of the alcoholic precipitate from any bacterial culture. Toluol was added to the hanging drop to prevent growth of the sporidia, but it is not readily miscible with water, and growth of the sporidia occurred in patches in the drop. There was evidently, then, a constant increase of the smut. The same difficulty was encountered when chloroform was used instead of toluol. Under these circumstances, it would be difficult to determine the actual effect of the enzymes on the living, growing sporidia.

The determination of enzymes by cultural methods. A. Pectinase. The following medium was found satisfactory for this test: Magnesium sulphate, 0.25 gm.; dibasic potassium phosphate, 0.25 gm.; agar, 15 gm.; distilled water, 1,000 cc. To this was added 1 per cent pectin. Both a slightly acid and a neutral reaction were used. The bacterium grew well at both reactions, coloring the medium brown. No nitrogen was supplied, so the organism evidently obtained enough of it from the impurities in the agar, although it does not grow on agar alone nor on the medium without the pectin. The pectin used was from lemon, and the reaction of the medium was adjusted with calcium carbonate and sodium hydroxide. It seemed possible that some citric salts may have been formed from an impurity in the pectin and that these might have supported the growth obtained. The bacteria failed to grow, however, when the pectin was replaced by calcium and sodium citrates; apparently, therefore, it is the pectin that supports growth.

B. Chitinase. In testing for chitinase Benecke's (3, p. 241) medium was used. This consists of agar, 20 gm.; distilled water, 1,000 cc.; magnesium sulphate, 0.35 gm.; dibasic potassium phosphate, 0.15 gm. To this were added pieces of sterilized chitin. It is best to add the chitin after the agar is cooled. If added before, it sinks to the bottom. It was prepared from hard-shell crab, according to Benecke's (3) method, as follows: After removal, the exo-skeleton was soaked in dilute hydrochloric acid for a few hours to remove all adhering particles of flesh. Then it was boiled in 20 per cent sodium hydroxide for about 8 hours and washed until the reaction of the washing water was neutral. It was finally washed in alcohol and ether. Commercially prepared chitin also was used. This gave an acid reaction, so some of it was used without adjusting the reaction, while in

other portions the reaction was brought to neutral. Bamberg's culture did not grow on any of these media. Evidently there is no chitinase produced by this bacterium.

C. Cellulase. The bacteria did not grow upon filter-paper or upon precipitated cellulose. Cellulase evidently is not present.

MORPHOLOGY OF BACTERIUM B–1

The bacterium is a gram-negative, nonmotile coccus. It passes through a coarse filter but not through the finer ones. In filtering, checks were made by mixing the culture with yellow chromogenic bacteria and with *Bacillus carotovorus,* but these were never found developing in the filtrate. The number of individual cocci going through the filter is very small, for the filtrate is always clear, and no growth is evident in it for 4 or 5 days. In an active culture the growth is slimy, each cell being surrounded by a capsule-like exudate. A capsule cannot be demonstrated by the usual staining technique, but a dilute solution of methylene blue (1–10,000) stains only the central core, leaving a halo around the individual bacterium. This pseudocapsule stains with the usual bacterial stains, therefore the organism appears larger than it really is. It is extremely variable in size, depending on the type of medium upon which it is cultivated. When stained with the usual bacterial stains, it ranges from 0.7 micron to 0.9 micron. The central core is about 0.4 micron. Old cultures are dry and powdery, resembling precipitated chalk. The cells often occur in chains of four or five individuals, and one cell is usually larger than the others in the chain. In extremely old cultures these large cells are very numerous, and chain formations no longer exist. This suggests contamination, but transfers to fresh media do not give rise to a contaminated growth. It probably indicates a certain amount of pleomorphism.

This bacterium is apparently very prevalent. It was obtained several times from contaminated oat-smut cultures supplied by C. S. Holton of the Department of Plant Pathology. It also was isolated many times by the writer from discolored areas on corn plants at the point of inoculation with corn smut. It apparently is specific for smut. It has no effect on species of Fusarium, Verticillium, Penicillium, Aspergillus, and many other types of fungi not identified.

CULTURAL CHARACTERISTICS OF BACTERIUM B–1

The bacterium grows readily on most of the media. It is always extremely viscous and grows anaërobically and aërobically. It produces gas and acid from dextrose and mannite. On sucrose it produces gas, but no acid; on lactose, acid and no gas. On litmus milk it grows with an acid reaction, the milk finally being peptonized. It does not liquefy gelatin and has no diastatic action. There is nothing distinctive about its growth

on potato plugs. As previously mentioned, it grows on pectin, with a brown discoloration of the medium. It reduces nitrates to nitrites with a pronounced reaction in the sulphanilic acid-alpha-naphthylamine test used, as recommended by the Society of American Bacteriologists.

DISCUSSION OF THE ANTIBIOSIS OF BACTERIUM B–1

It would be difficult to decide just what factor is responsible for the destruction of the smut by bacterium B–1. The fact that the bacterium produces pectinase and the possibility of the presence of pectin in the cell wall of smut sporidia, especially in young sporidia, suggest that the cell wall may be dissolved. This is somewhat corroborated by the fact that débris and oil-like globules are present in hanging drops of a suspension of the sporidia and the bacteria. The destructive action of the bacterium on the young growth and not on the old also suggests some activity of the pectinase. As mentioned in the foregoing discussion, the old sporidial growth contains a preponderance of chitin and a substance staining blue with dilute methylene blue, which possibly may be cellulose or some closely related substance. Since neither cellulase nor chitinase was found in the organism, it is not likely that it produces any enzyme that could act on these substances. However, it is evident that the presence of pectinase alone does not account for its antibiotic action. Other bacteria, such as *Bacillus carotovorus* and *B. mesentericus,* have no destructive effect whatever on corn smut. On the other hand, Bamberg's bacterium does not produce a soft rot of carrot. Jones (9) concluded that in soft rot there was involved an "active osmotic substance." Evidently, each of these two organisms has its own specific antibiotic property. Possibly the destructive action of the bacterium in question cannot be ascribed to any one factor but may be the result of the interaction of a group of factors. Frost (6), in 1904, made a study of the antagonism of certan soil saprophytes to *B. typhosus.* His conclusions are probably applicable also to the results obtained by the writer on Bacterium B–1. They are, in part, as follows: 1. "The antagonism results in not checking the growth, but in actually killing the typhoid germs. In many cases the killing amounts to extinction." 2. "There is no evidence to show that the antagonistic substances exist ready-formed in the soil, but rather that the antagonism depends on the rapid development of the germs in the immediate presence of *B. typhosus.*" 3. "Changes in the environment of these organisms, such as temperature, oxygen supply, reaction of medium, amount of dextrose, etc., seem to have little or no influence on the production of the antagonistic substances. In other words, whenever the environment is such that a good growth of the organism occurs, the antagonistic substances are always produced." 4. "The cause of the antagonism is not due in the cases studied to the exhaustion of food supply, the action of

proteolytic enzymes, specific poisons, or the production of the hydroxyl ions supply."

Bamberg thinks that the organism B–1 may be of some economic importance, inhibiting the smut infection of corn plants to some extent under natural conditions. Whether it be of economic value or not, a study of it gives interesting information in regard to processes of antibiosis that are probably going on in nature.

OTHER BACTERIA ANTIBIOTIC TO SMUTS

In the course of some work on the black-chaff bacteria, the writer found several other types of bacteria that are antibiotic to smuts. As previously mentioned, these are designated by their laboratory numbers as bacterium C–1, D, and Myxobacterium–1.

Bacterium C–1

Bacterium C–1 is a small gram-negative rod. As far as it was studied, it is identical with Bacterium B–1 in its physiological reactions. It has a pseudocapsule, readily seen when dilute stains are used. It is constant in size; including the pseudocapsule, it is about 0.8 x 1.2 microns. The central core is about 0.5 x 0.8 micron. The growth is slimy, but it does not have the high degree of viscosity found in bacterium B–1. It grows upon the pectin media used for B–1. Alcoholic extracts were made similar to those made with B–1, but they had very little effect on carrot slices. The bacterium reduces large colonies of corn smut and of the oat smuts to a slimy mass with complete destruction of the sporidia. No other smuts were used in combination with it. Like Bamberg's culture B–1, its virulence is reduced by long-continued cultivation upon artificial media. When water suspensions of cultures of known virulence are inoculated into corn-smut galls, the latter are broken down and the young galls often turn green and disappear during growth of the plant. Like Bamberg's bacterium B–1, virulent cultures of C–1, when used in high concentrations in combination with sporidial suspensions, usually prevent gall formation in corn plants. It has no antibiotic effect on species of Fusarium, Penicillium, Aspergillus, Verticillium, and other soil fungi of unknown identity. It does not prevent the growth of fungi on the surface of soil moistened with broth cultures of it. It is, therefore, evidently specific for smuts.

Bacterium D

Bacterium D was found frequently as a contamination on agar plates by the writer in the course of some isolations of black-chaff bacteria. Contaminating colonies of corn smut and this bacterium were often present on the plates, and wherever the colonies of the bacterium came in contact with the smut colonies the bacteria overran the smut colonies and finally de-

stroyed them. Scores of smut cultures were inoculated with this bacterium and always with the same destructive effect. In the course of 10 to 14 days the sporidia in smut colonies an inch or more across were completely replaced by the bacteria. The colony retained its original shape and contour, the only change in the appearance of the smut growth being the slight discoloration as the bacteria advanced. Figure 1 shows a smut colony so

FIG. 1. Colony of *Ustilago zeae*, inoculated with bacterium D.

replaced by the bacteria, and Figure 2, A, shows a stained mount of the colony at this stage. For comparison a mount of only sporidial material at the same magnification is shown (Fig. 2, B). Like the previously discussed cultures, bacterium D does not affect the old smut growth. This is not because of the dryness of the smut culture, since the addition of sterile water at the time of inoculation did not increase its antibiotic effect.

Inoculations were made into young corn plants with a water suspension of sporidia mixed with a water suspension of the bacterium. Only one of three dozen plants developed galls, while 100 per cent infection occurred in two dozen plants, inoculated with sporidia only. The infection in that one plant may have been due to a survival of one pair of the proper strains of sporidia rather than to the inactivity of the bacterium. Another set of plants was inoculated to check this experiment, but no conclusions could be drawn from them, because no infection occurred in the checks.

Attempts also were made in the greenhouse at temperatures ranging from 14° to 20° C. to determine the effect of the bacterium upon the infection of wheat and oats by the various smuts. Inoculations were made both with cultures and with chlamydospores of the smuts and the soil was kept moist with bacterial cultures. Results were inconclusive, since no smut

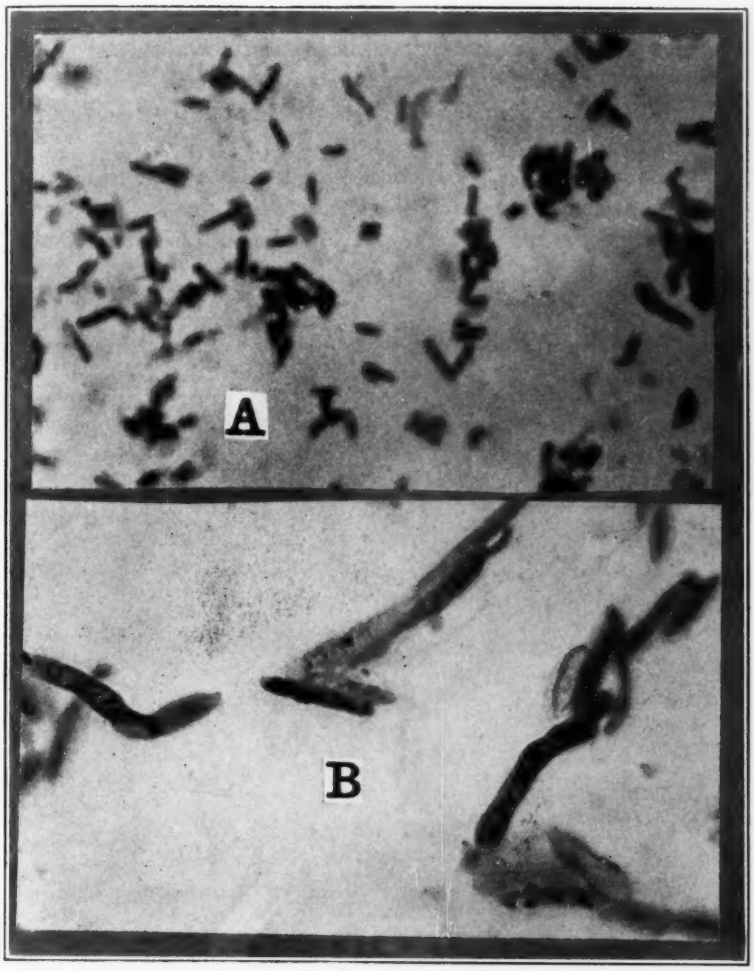

FIG. 2. A. Stained mount from the colony of *Ustilago zeae* shown in figure 1. The sporidia have been replaced by the bacteria. (×1,200.) B. Stained mount from sporidial material. (×1,200.)

developed in the checks. Rodents and birds repeatedly destroyed the plantings outside, so no idea was obtained as to the rôle of the organism in the infection of wheat and oats by smuts.

During some inoculation experiments to determine whether the organism can prevent bunt and oat smuts from infecting the seedlings in the soil, there was noticed the complete absence of Pyronema and other fungi from the tops of the pots kept moist with the broth cultures of the bacterium. Without exception, the checks, to which only sterile broth was added, were

covered with fungus growth. To check further on this observation, a series of pots of soil was used in another experiment in which one set was kept moist with a broth culture of the bacteria, another set being moistened with broth without the bacterium. The former remained free from fungus growth, while the latter was covered with it. In another set of pots inoculations were made with various fungi found growing on soil; some were moistened with a broth culture of the bacterium and some with broth only. The results were the same as in the previous series. Figure 3 shows a pot

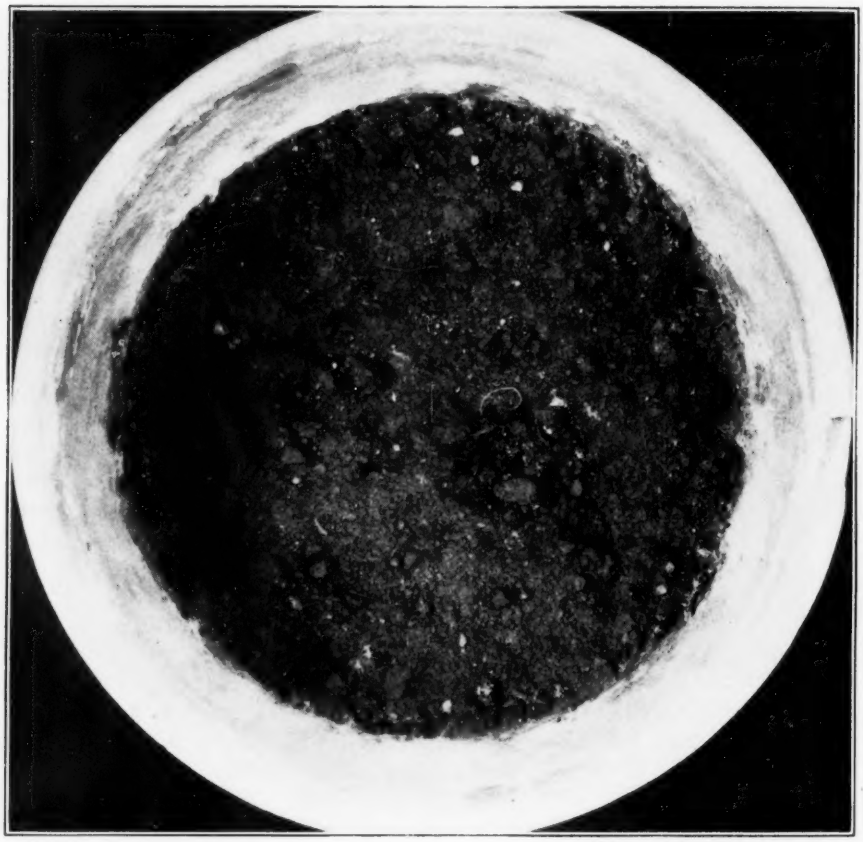

FIG. 3. Soil inoculated with a mixture of soil fungi, moistened with a broth culture of bacterium D, and showing the absence of fungus growth.

so inoculated with fungi and moistened with the broth culture of the bacteria. The surface shows no fungus growth. Figure 4 shows a check, a pot inoculated with fungi and moistened with broth alone. It is overgrown with Pyronema and Penicillium. Evidently, bacterium D is active in the soil and has an antibiotic effect upon some soil fungi.

FIG. 4. Check: Soil inoculated with approximately the same amount of soil fungi, moistened with growth without bacterium D, and showing growth of Pyronema and other soil fungi. Compare with figure 3.

No enzymes of any kind could be demonstrated in bacterium D either by cultural methods or by precipitation of broth cultures with alcohol.

Specificity. A. In artificial media. The organism destroys colonies of *Ustilago zeae, U. levis,* and *U. avenae.* It was not tried on other smuts. It also kills one species of Penicillium. It remains in a vegetative condition for a long time in liquid cultures of other species of Penicillium but is finally crowded out. This may be due to a difference in rate of growth. It does not affect species of *Verticillium* and *Fusarium.*

B. In soil. As previously stated, no conclusions could be drawn from the inoculations of the smut and the bacterium into the soil because no smut occurred on the checks. However, as shown, the growth of many types of fungi was inhibited. Possibly the high buffer content of the soil protects the bacterium against excessive acid. In a liquid medium excessive

acid is unfavorable to the growth of the bacterium and produces sporulation.

Cultural characteristics. The bacterium produces no gas. It produces acid on dextrose but not on lactose, sucrose, or mannite. It has a proteolytic action on milk and liquefies gelatin rapidly. It does not produce indol. It has no distinctly characteristic growth on potato and does not produce diastase. It is not chromogenic. Under certain conditions it grows in a star-shape colony. It probably belongs to a group of spore bacteria with this same characteristic, for two other cultures were isolated with this same type of colony but they differed from this bacterium in their sugar reactions and had no destructive effect on smut.

Morphological characteristics. The bacterium is a motile spore bearer. It is variable in size, depending on the richness of the medium, ranging from 0.6 x 2 to 1 x 3 microns. On media rich in dextrose and protein it assumes very bizarre shapes. It is somewhat pleomorphic, the rod-shape forms often breaking up into round bodies which stain with the common bacterial stains. The cells usually occur singly, but short chains may be found. It is gram-positive in young cultures, but the cells gradually lose this property in old cultures.

Discussion of the antibiosis of bacterium D. On sugar media the bacterium sporulates quickly, but, if smut is added to a broth culture reduced almost completely to spores, the culture starts growing and may continue in the vegetative form for weeks. The same is true when inoculations are made with certain species of Penicillium and other fungi. It may be possible that the fungi utilize acids and other metabolic substances that would otherwise cause staling of the bacterium. Hill (8) found that certain species of Penicillium activated cultures of certain luminous bacteria. When filter-paper medium was inoculated with the bacterium D no growth occurred. Finally the medium became contaminated with a Penicillium, which grew upon the inorganic salts solution and spread over the filter-paper without affecting the paper. Wherever the Penicillium grew there was abundant growth of the bacterium, and it continued to exist in the vegetative form. The bacterium destroys the sporidia in smut colonies an inch or more in diameter in 10 to 14 days. The smut colony retains its shape and general characteristics, the only indication of the advance of the bacterium being a slight change in color of the smut colony. The bacteria replacing the smut culture are in the vegetative form as long as there is smut left. The bacteria on the agar medium surrounding the smut are in the spore condition after a few day's growth. In the broth culture the antibiotic effect on the smut is not so pronounced; the bacterium prefers aërobic conditions, and the corn smut grows well under semiaërobic conditions. This may hamper the bacterium to some extent. Then, too, the

bacterium is sensitive to acid. The agar, with its high buffer content, probably protects it against this staling product, enabling it to continue its growth upon the smut, while the broth does not contain so much buffer material. The inhibition of fungus growth upon pots of soil inoculated with fungi and moistened with the bacterial culture suggests the same thing, that the buffers in the soil probably maintain a favorable reaction for the bacterium. It seems evident that, when other conditions are suitable, the bacterium obtains some element of food from the fungi to which it is antibiotic.

It is difficult to suggest what the antibiotic substance is. Sporulating cultures that had been boiled vigorously for 10 minutes, then allowed to germinate, did not lose the antibiotic property. As stated before, no enzymes were demonstrated that could dissolve the cell walls of the sporidia. It produces no acid which might be destructive to them, for the filtrate of the bacterial culture supported growth of the smut. Its virulence is destroyed by constant cultivation upon artificial media. With all findings negative, it would seem again that the antibiotic action may be due to a factor or a group of factors that are not readily demonstrated.

Myxobacterium–1

A Myxobacterium, also, was found by the writer in the course of some work on black chaff. It appeared as a pink streak in a corn-smut colony contaminating an agar plate. At first it was thought to be a Fusarium and was kept under observation to see which one would crowd out the other. Further study showed that it consisted of rod-shape bacteria and round spores, the latter forming globular or somewhat oval fruiting bodies. This development corresponds to that of the Myxococcus, a genus of the Myxobacteria.

According to Quehl (16) and several other investigators, Link described a Myxobacterium in 1795, but he thought it was a Gasteromycete. Schroeter recognized the bacterial nature of the organism and described two species in 1889. However, he made no further study of them. Thaxter (18, 19, 20, 21) made the first extensive study of the group. This work was followed by a series of articles by European workers, among whom are Baur (2), Kofler (10), Quehl (16), and Vahle (23). More recent work has been done by Helena Krzmieniewska and S. Krzmieniewski (11, 12, 13) of Poland. Emoto (4) of Japan recently has published a list of the investigations on the group up to date.

Faull (5) has summarized the characteristics of the myxobacteria as follows: "The Myxobacteriaceae constitute an extremely interesting assemblage of forms because of their apparent relationship to the bacteria on one hand, and to the slime molds, particularly the Acrasieae, on the other. The

individual plants are bacteria-like rods in all cases, and after a vegetative period, these swarm together to organize a definitely shaped pseudo-fructification, without, except in Myxococcus, undergoing any marked morphological changes themselves. These fructifications are not comparable to the more or less heaped-up colonies characteristic of certain bacteria; but, as Thaxter has pointed out, are strictly comparable to the fructifications of the Acrasieae."

Myxobacteria have been isolated from many sources, such a dung, rotting wood, decaying leaves, lichens, fungi, and filter-paper, and once from a bird's nest. Thaxter (18) in 1892 described one of them, a species of *Chondromyces,* as "parasitic upon living lichens, which it destroys." Geitler (7) in 1924 isolated a species of Polyangium, which invades the cells of a Cladophora species and destroys this alga.

Myxobacterium–1. As previously mentioned, this culture was first observed growing on a smut colony contaminating an agar plate and appeared as a pink streak in the smut growth. It evidently belongs to the genus Myxococcus. It consists of a rod-like, often slightly curved, vegetative form, and in its later growth produces round spore-like bodies which form globular or slightly oval fruiting bodies. Among the species of Myxococcus described, it resembles *Myxococcus rubescens* Th. more than any other. However, Vahle (23) and, later, Yoshi (25) have reported that *M. rubescens* liquefies agar. Since liquefaction has not been obserevd in the case of Myxobacterium–1, it does not correspond entirely to the description of *M. rubescens.* So far, the work on the myxobacteria deals with the source, method of isolation, and the morphological characteristics of the group. Until a comparative study, including the physiological characteristics, can be made, it would not be possible to identify the species discussed here. So much work has been done on the morphology of the group, the most recent being on those species found in Poland, that no general discussion is necessary.

Myxobacterium–1 is colorless in its vegetative stage, but, when the pseudoplasmodium begins to draw together to form fruiting bodies, the colony often acquires a pink tinge. The fruits range from a faint to a dark pink. Several times they have turned to a brick-red, when growing on old smut growth. Fruiting bodies are formed much more rapidly on drying media, although they finally form even in a liquid medium. Preliminary to fruiting the vegetative mass evidently seeks the high, dry places, wherever this is possible, for the fruits are usually found on elevated areas. Figure 5 shows fructifications on the ridges and protuberances of old smut growth. On chitin the fruits almost always appear at the apices of the pieces. Figure 6, A, shows the lateral view of the characteristic position on chitin.

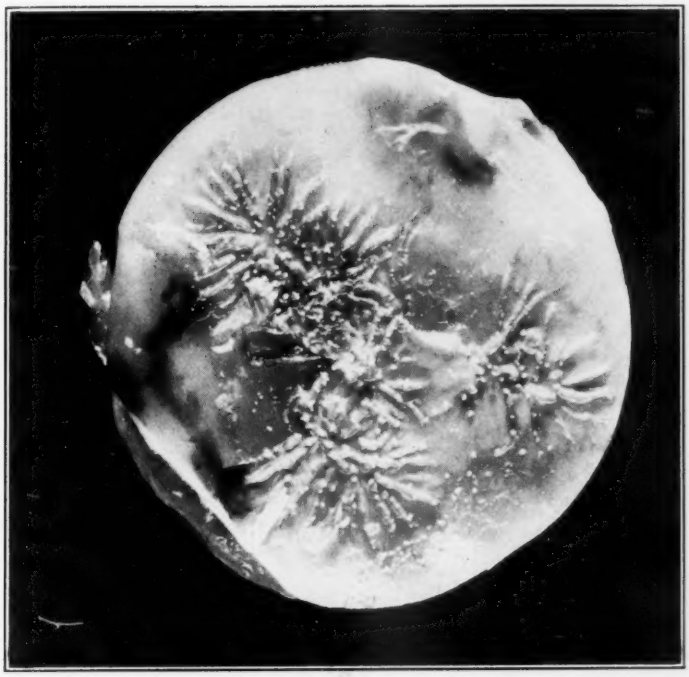

FIG. 5. Large colony of *Ustilago levis* with the fruiting bodies of Myxobacterium-1. (Natural size.)

Cultural characteristics. The best medium for this Myxobacterium is young, vigorously growing corn smut or the oat smuts. The smut becomes covered and permeated with the colorless vegetative growth of the organism. In about 10 days fruiting occurs, the smut colony being covered with pink, bead-like fruits, placed on the elevations of the smut. These may remain intact for several months. The bacterium grows well on Kofler's cane-sugar medium of the following composition: Peptone, 2.5 gm.; cane sugar, 15 gm.; magnesium sulphate, 0.25 gm.; agar, 9 gm.; tap water, 500 cc. It also grows well in a liquid medium of the same salts and organic constituents. The following precipitated cellulose medium is very favorable: Ammonium magnesium phosphate, 2.0 gm.; dibasic potassium phosphate, 0.25 gm.; magnesium sulphate, 0.25 gm.; sodium chloride, 0.1 gm.; ferric chloride, trace; agar, 15 gm.; precipitated cellulose; tap water, 1,000 cc. This gives a slightly alkaline reaction, which seems favorable to the organism. At times a very scant growth has been observed on the medium without the precipitated cellulose, but the difference in the amount of growth is so marked that it can undoubtedly be assumed that the precipitated cellulose serves as a food element. It also grows readily on filter-

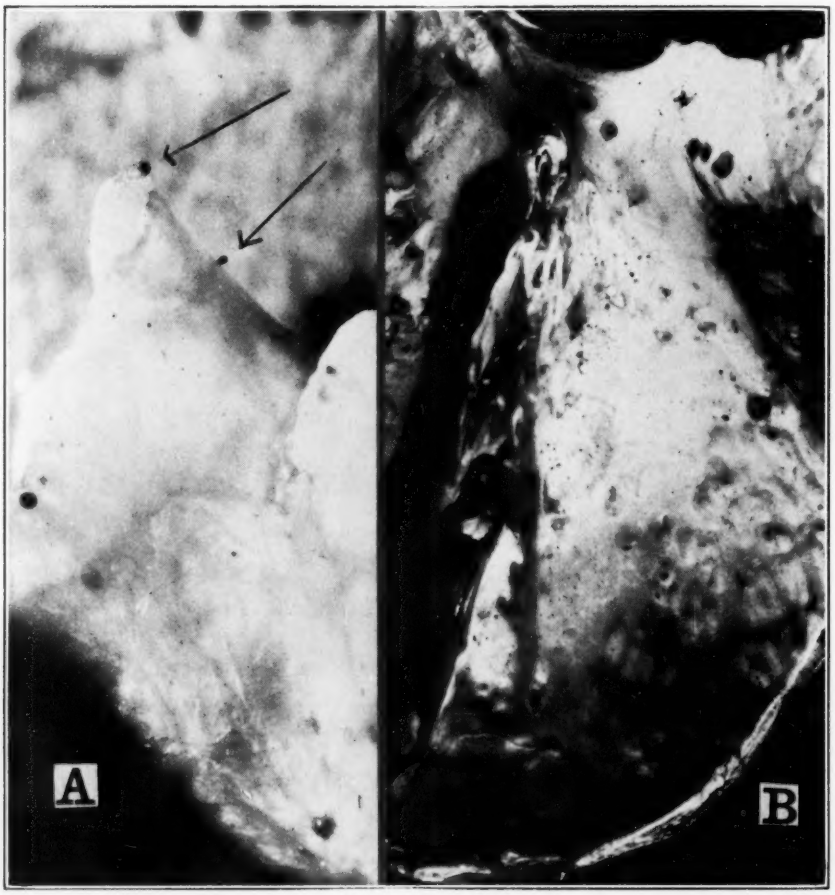

FIG. 6. A. Chitin pieces showing fruiting bodies of Myxobacterium-1 in characteristic position on the higher portions of the medium. (×6.) B. Fruiting bodies of Myxobacterium-1 scattered over a piece of chitin. The chitin was originally white, but it has been darkened by the bacterial growth. (×6.)

paper medium, consisting of the salts used in the precipitated cellulose medium, 1 per cent agar, and shredded filter-paper of a good grade. Shredding is accomplished by the use of a nutmeg grater. The paper absorbs so much of the water in the medium that it becomes too dry to support growth if more agar is added. It does not grow on filter-paper of the same grade when this is suspended in a liquid solution of the same salts. If the suspended filter-paper is inoculated with both the bacterium and the corn-smut sporidia, the latter grow fairly well on the liquid medium and the colonies spread over the moist filter-paper. These smut colonies usually are centrally pink, as a result of the growth of the Myxobacterium on the smut.

The organism also grows fairly well upon the pectin medium that was used in culturing bacterium B–1 and C–1, turning the medium a dark brown. It grows upon the chitin medium previously described, turning the chitin a dirty pink and gradually softening it. Figure 6, B, shows the fruiting bodies scattered over a piece of chitin. The chitin was originally white and has been blackened by the bacterial growth. Sterile water must be supplied often enough to keep the chitin moist or no growth takes place. Growth on chitin is slow, several months being required before any perceptible change occurs. No further study of the food requirements of the bacterium was made.

Effect on smut growth. When the bacterium is inoculated into young corn-smut cultures, the fructifications occur, as previously mentioned, on ridges and elevated portions of the smut. Examination with a hand lens shows that the protuberances are levelled down, so the part overrun by the bacterium has a smooth surface, while the areas that are not overgrown have a rough surface. The bacteria confine themselves to the smut, never spreading beyond it upon the media.

Specificity. The organism grew upon corn smut and the oat smuts. It was crowded out by Penicillium, although fruiting bodies of Myxobacterium frequently were found among the hyphae of Penicillium. No further study was made of its specificity nor was its economic importance determined.

Discussion of the antibiosis of Myxobacterium–1

Myxobacterium–1 has not been found within the smut cells, so its action must be extracellular only. It evidently is able to produce chitinase, pectinase, and cellulase. If it be a fact that the cell walls of the corn and oat-smut sporidia contain chitin, pectin, and cellulase, or some closely related substance, as microchemical tests suggest, then this bacterium may be able to attack and dissolve the cell wall of the sporidia. However, as previously mentioned, not all bacteria producing pectinase are antibiotic to smuts. Similarly, not all bacteria that produce chitinase have a destructive effect on the smuts studied. For example, another species of Myxococcus and an Actinomycete were found which break down chitin but which have no effect whatever on smut. Evidently, then, the presence of the enzyme is not the determining factor. If it play a part in the antibiosis, there must still be some other factor involved which has not been demonstrated in the course of this investigation. Probably the outstanding result of this study is the evidence obtained that several groups of bacteria are concerned in such antibiotic processes going on in nature.

SUMMARY

1. Four types of bacteria are discussed which are antibiotic to certain smuts and other fungi. They consist of a coccus; a motile, nonspore-bear-

ing, rod-like bacterium; a motile, spore-bearing, rod-like bacterium, and a Myxobacterium.

2. A study of the enzymes of these bacteria was made to determine if they can break down the cell wall of the sporidia and so destroy the smut growth.

3. Sporidia of corn smut were stained to determine the chemical constituents of the cell wall.

4. Some of the bacteria contain enzymes with which they may be able to dissolve the corresponding chemical constituents of the cell walls of sporidia.

5. However, certain other bacteria, with the same types of enzymes, do not affect the sporidia of the same smuts. There are, therefore, probably other factors involved in the antibiotic property.

6. The cultures become avirulent after long cultivation upon artificial media.

7. Some experimental work was done on all the cultures except the Myxobacterium to determine their effect upon infection of corn plants by smut. The results indicate that they may inhibit infection to some extent under proper conditions.

8. While it is impossible to make any statement as to the economic importance of this antibiosis, the study suggests that antibiotic processes occur in nature.

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YELLOW SPOT OF PINEAPPLES IN HAWAII

J. F. ILLINGWORTH[1]

INTRODUCTION

A destructive disease of pineapples, new to science, has demanded during the past 4 years the serious attention of the scientific staff of the Experiment Station of the Association of Hawaiian Pineapple Canners, in Hawaii. Observed first as a distinct disease, in the spring of 1926, narrow in its range of distribution, it has since become wide-spread and at times exceedingly destructive.

This paper is designed to present a picture of the nature of the disease, giving a history of its development, with observational and experimental data leading to the discovery of the probable vector, a species of thrips. Only tentative control measures are suggested. Further study of the problem is in progress by other workers.

Dealing with this disease, Dr. C. P. Sideris made the first studies.[2] During the next 2 years the disease assumed alarming proportions, and Mr. Glenn E. Paxton reported fully,[3] with excellent illustrations, summing up our knowledge of the subject to date, at that time. Both papers were published only in the local organ of the pineapple industry.

DESCRIPTION OF THE DISEASE

The first appearance of this disease, the so-called "initial spot," is a slightly raised yellowish spot on the upper surface of the leaf. It varies in size from $\frac{1}{8}$ to $\frac{1}{2}$ inch in diameter. When fully developed the darker center is surrounded with a halo of yellow (Fig. 8). Ordinarily only 1 leaf on a plant is thus affected, but in extreme cases we have found as many as 5 initial spots on a single plant. When the spot makes its appear-

[1] This study has not been an individual effort. It was carried on jointly by the Entomological and Pathological Departments of the Station. Mr. Glenn E. Paxton, of the latter Department, cooperated with the writer closely in all of the later field work. Furthermore, it was his suggestion in April, 1929, that side rot might be a virus disease, which started us on the right track to our goal. Dr. Royal N. Chapman, head of the Department of Economic Entomology at the University of Minnesota, also was associated with the investigation for a brief period, during October and November, 1929. He made a most important contribution to the investigation, discovering the characteristic, microscopic, insect punctures associated with the yellow spot on the leaves. Finally, the work of Dr. M. B. Linford, of the Pathological Department, indicated that the vector was an insect of very small size, which embedded its egg in the pineapple leaf. It was upon the basis of this work that the vector was suspected to be a species of thrips.

[2] Side rot of pineapple plants, March, 1927.

[3] "Side rot" or "yellow spot" disease, June, 1929.

ance it is 3 to 8 inches from the base of the leaf. This is due to continued basal growth during the fairly long incubation period. After infection takes place, in the axillary region of the leaf, 10 to 20 days must elapse before the yellow spot is apparent. The rate of growth of the individual plant determines the distance up on the leaf. All the evidence goes to show that the insect infection takes place near the center of the plant, for the initial spots appear on the leaves of the third or fourth whorls from the center.

FIG. 1. Young pineapple plant suffering from an advanced stage of yellow spot. Note the characteristic tipping of the plant to the right. The arrow points to a spot on a leaf, where infection took place. Photograph of colored plate by Armena Eller.

Under favorable conditions, a yellow streak develops directly below the initial spot, widening in the region of the white tissue at the base of the leaf. The tendency of this streak is to become constricted into circular yellow blotches, giving it the appearance of a chain of beads. These usually start an inch or more below the initial spot. After a few days the portion of the streak in the white tissue, at the base of the leaf, has a water-soaked appearance. In the presence of moisture in the leaf axils, rot soon follows.

extending to the stem. A few days later, a yellow streak, developing into the characteristic bead-like chain, can be observed extending up the next leaf above the one first affected. This usually spreads quickly to the other central leaves, and the whole plant is doomed.

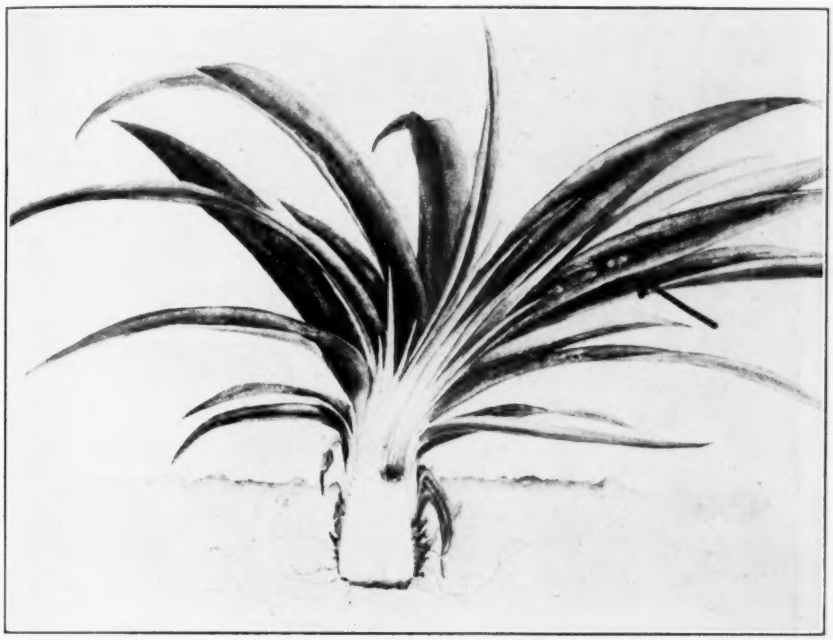

FIG. 2. A median section of the plant shown in figure 1. Note how the rot is progressing downward to the stem. The initial infection on the leaf is indicated by the arrow. Photograph of colored plate by Armena Eller.

At the point where the tissue of the stem is affected, the stem ceases to grow. The normal development of the healthy part, on the opposite side, soon causes the plant to bend over very decidedly (Figs. 1 and 2). This led at first to the name "side rot" to designate the trouble.

Side rot is primarily a disease of young plants. Of the three types of planting material, tops are by far the most susceptible because of their loose structure, permitting the vector easy access to the tender tissue in the leaf axils. Tops, while still attached to the fruit, may also be affected (Fig. 3). This usually follows in a field that has previously suffered from the disease on the plants. Yet, in several instances, it occurred first on the foliage of the fruits, in fields where the disease had not troubled the plants.

History of its development. This disease first came to my attention May 24, 1926. It appeared in a young field on Oahu, located in a fairly

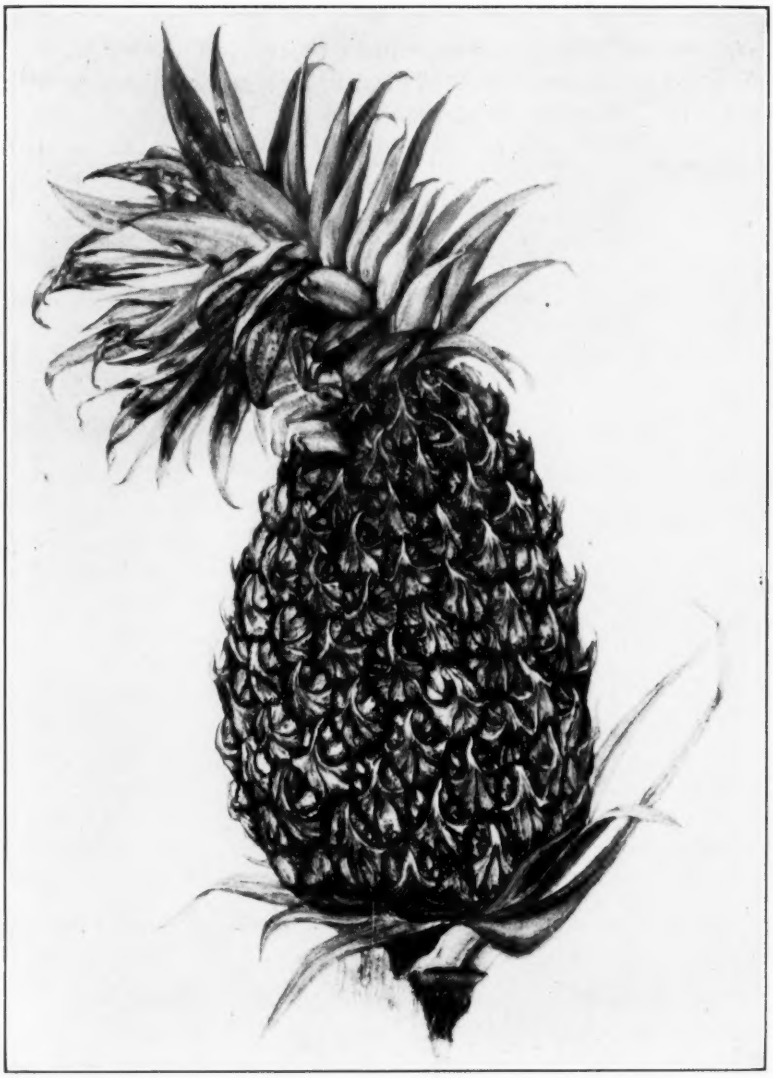

FIG. 3. An advanced stage of yellow spot, showing its effect upon the top. Here most of the leaves had died and rot was progressing downward into the fruit. Photograph of colored plate by Armena Eller.

rainy section. After the warm weather came on, in June, the trouble ceased. That was an exceptionally dry year. With cooler weather and occasional showers in December, the tops on the fruiting pineapples in this same locality began to suffer from the disease, resulting in the rotting of many of the fruits.

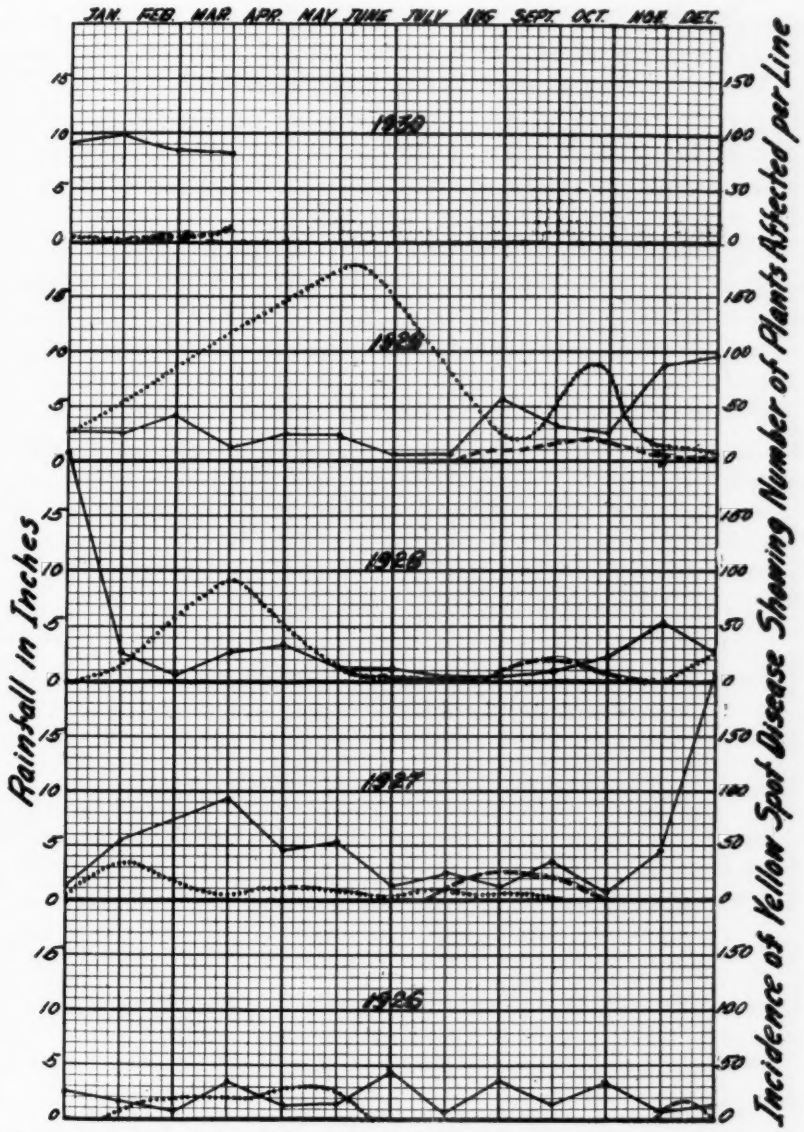
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC.
1930
1929
1928
1927
1926
Rainfall in Inches
Incidence of Yellow Spot Disease Showing Number of Plants Affected per Line

FIG. 4. History of yellow spot in relation to rainfall. Rainfall indicated by solid lines; virulence of disease on young plants, by dotted lines; on tops (attached to fruits), by dashes. The rainfall records are those of Schofield Barracks in the heart of the pineapple section on Oahu and are fairly representative of the fields from which the disease data are recorded.

The season of 1927 opened with increased rainfall (Fig. 4) and the disease made rather slow progress. A new outbreak, however, extended the range to a distance of several miles, into a fairly dry region. The disease also appeared on tops of fruits in a field planted with suckers. This indicated that we were dealing with a new primary infection.

The spring of 1928 was a period of few intermittent rains and the trouble increased materially over what it had been in the 2 previous years. It not only was more virulent in the original locations but continued to spread to more distant centers. However, the trouble ceased as hot, dry weather came on at the end of May. The disease began to appear again in December after the rainy season started. We were at a loss to know the cause of the trouble. Sick plants were pulled and carted out of the fields by dray loads.

The year 1929 opened with low rainfall, and the disease again developed with increasing virulence (Fig. 4). The spread was tenfold what it had been in previous years. It now appeared in practically every section where pineapples were grown on Oahu. Furthermore, it continued into the summer; in fact, it never completely disappeared, for diseased plants could be found throughout the hottest and driest weather. The trouble appeared to be slowed down only by these adverse conditions. It now became evident that the disease was systemic. In fields hit the previous year, we found all types of planting material while still attached to the parent plant, showing the characteristic yellow spots, with leaves rotting at the base. Tops, slips, and suckers were affected alike. Evidently the parent plant had carried the trouble over from the previous season.

In June, 1929, the disease was reported from the eastern end of Molokai, probably taken over on the planting material from Oahu. We made a careful survey and found conditions very similar to those on the island of Oahu.

In September, 1929, there was an exceptionally early outbreak of yellow spot in a young field, located near the original center of the trouble on Oahu. Virulence increased until rains set in, when the disease disappeared as suddenly as it came (Fig. 4). Here the weather seemed a very important factor. During November, 1929, this disease was found to be fairly widely distributed on Maui. This left only the islands of Kauai and Hawaii unaffected.

Observational and experimental data. As I have indicated, climatic conditions evidently play an important part in the activity of the disease. The relation to rainfall is clearly shown in the graph, figure 4. Even after the initial spot makes its appearance further progress of the disease is determined by weather conditions. Humidity hastens the disease, while drought slows it down for months.

Long before we knew what was causing the disease, we began to notice a very definite relation to the prevailing winds coming from old fields. A good illustration is that on east Molokai: in this case, the infected area was limited and appeared to have a very definite relation to the prevailing winds coming from old weedy fields in which tops were badly affected with the disease (Fig. 5). We could not find a trace of the trouble anywhere else on Molokai. Young fields lying not more than a quarter of a mile away from this infested center showed perfect growth.

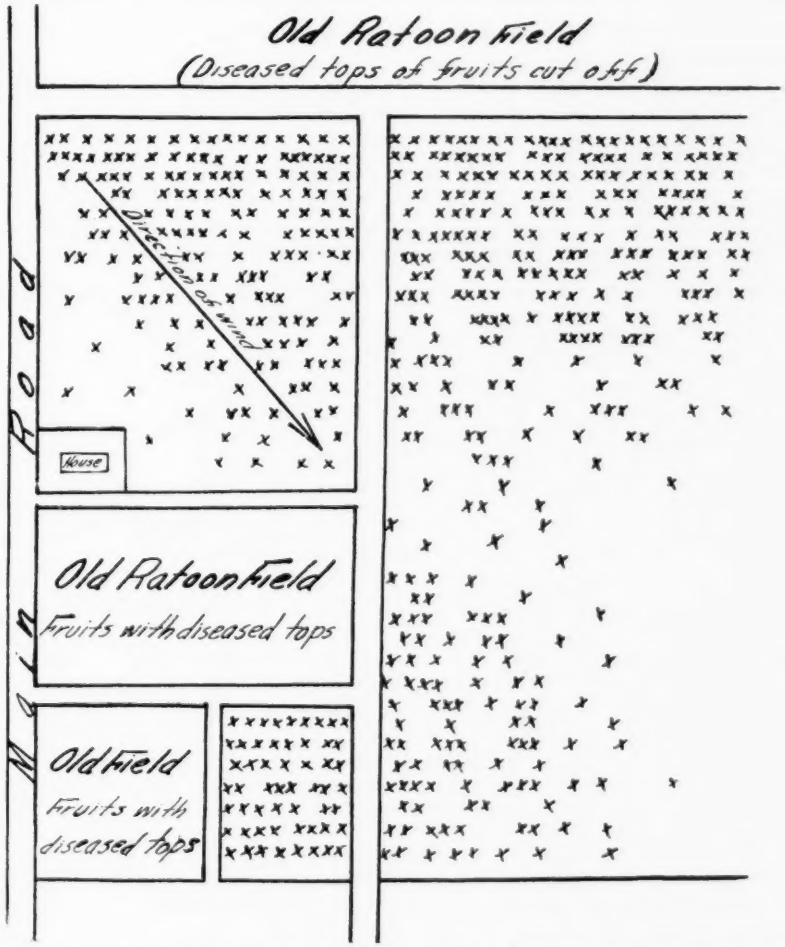

FIG. 5. Sketch to show the relation of infection of yellow spot to prevailing winds on Molokai. The old fields were very weedy, and most of the pineapple tops had been cut off, because they had been attacked by this trouble. Diseased plants are indicated by × marks (each "×" representing several plants).

This wind relation I had found in evidence in several places on Oahu. As a typical example I will cite a field of 77 acres at Kunia, which is a fairly arid section (Fig. 6). The windward area of this field suffered serious injury from yellow spot. Careful counts of plants demonstrated that the disease gradually diminished toward the leeward side, where there was no evidence of the trouble whatsoever.

Mr. Paxton and I began a study of field weeds early in April, 1929, following his suggestion that this might be a virus disease with some weed as a host. At that time we decided to call the trouble "yellow-spot" dis-

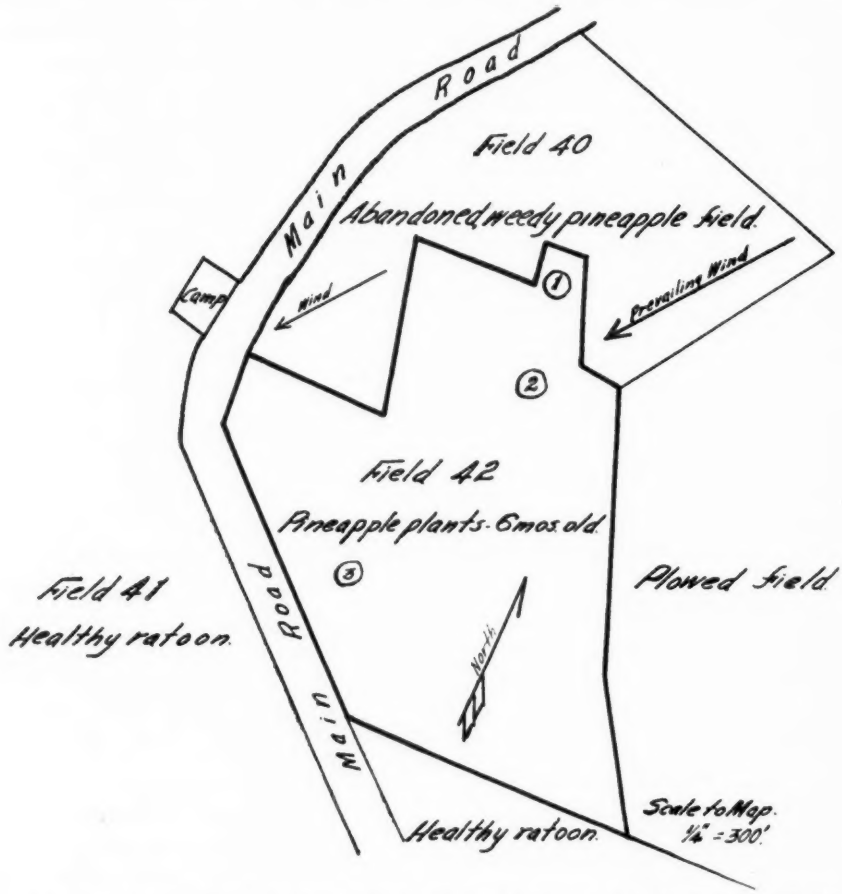

FIG. 6. A 77-acre field at Kunia on Oahu, which showed a decided wind infection, from an abandoned, weedy area adjoining. In the portion of the field marked 1, the loss from yellow spot was extremely heavy. A little further in, 2, the losses were not more than one-half as much, while at the leeward side, 3, there was no indication of the trouble.

ease, because of the confusion in the use of the name "side rot" with several other rots on pineapples.

We collected all the various insects found on weeds in the affected fields, to determine what vector was transmitting yellow spot. Preliminary trials were made with the following insects, enclosing them in large lantern chimneys over pineapple plants, both in the field and in our greenhouses: (1) Nysius bugs, from Erigeron, Portulaca, and Bidens; these bugs were observed frequently down in the heart of young pineapple plants, especially following weeding of the fields. (2) Plant lice, from Sonchus, nightshade, Portulaca, Emilia, and several other weeds. (3) Jassids, from nightshade and Paspalum grass. (4) Thrips, from Emilia flowers and leaves. Results from these preliminary trials were all negative, as were likewise our numerous attempts to reproduce the disease artificially.

Dealing with susceptibility to yellow spot, I critically studied the various types of planting material, especially as to structure and its effect upon insect population.[4] Even a casual observation indicated that tops, due to

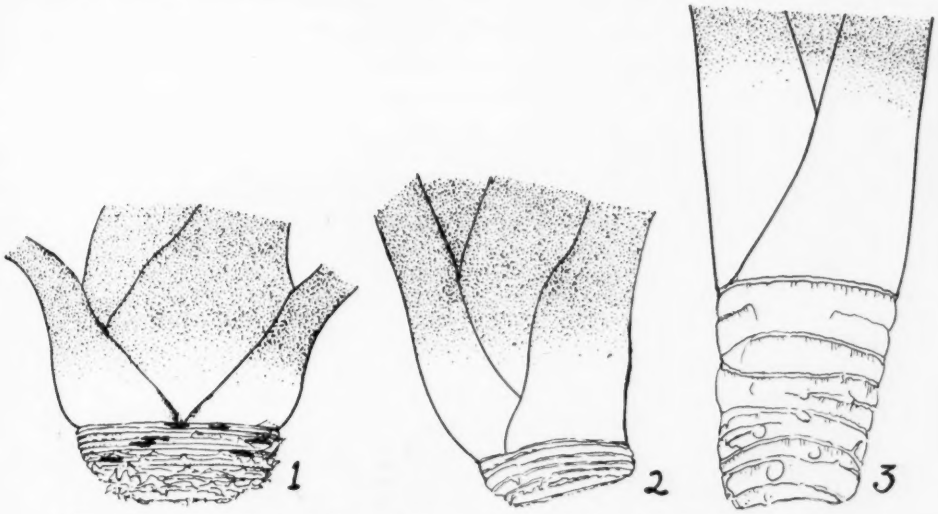

FIG. 7. Sketches of the butts of stripped Cayenne planting material. 1. A crown showing leaves loosely attached and not coming together at edges. Here we see discoloration, where insects, mites, etc., are usually secreted, feeding on the very tender white tissue. The dark spots on the stem are the early stages of injury by the fungus Thielaviopsis, initiated by these pests. 2. A slip showing overlapping of the leaves at their edges. Due to the fact that they clasp the stem more tightly, the white area at the base is considerably greater than seen in the crown. 3. A sucker, showing extremely tight imbrication of the leaves about the stem. It is practically impossible for thrips to infect this type of plant. (Engraving, courtesy of Experiment Station, Association of Hawaiian Pineapple Canners.)

[4] Leaf characters and resistance to yellow spot. Reported on in Station files for October, 1929.

their open, spreading leaves, have a much greater insect population than suckers (Fig. 7). The closer imbrication of the leaves in the latter evidently accounts for their relative immunity from yellow spot.

During the period July to September, 1929, further experiments were carried on with possible vectors. Thirteen kinds of insects were used. Besides those studied previously, all of the following various organisms found in my study of structure of pineapple planting material were now included: Tarsonemus mites, red spider, and a species of thrips that rather constantly occurs with them, pineapple mealy bugs, spring tails, bud-moth caterpillars, Chironomid maggots, Nitidulid beetles, and *Scolia manilae* wasps. All of these are closely associated with the growing pineapples, but none of them gave positive results.

The quest for the vector was now taken up on a much-increased scale. Plant lice were again studied thoroughly. Jassids of two species, rather abundant on weeds in the affected field, were caged by hundreds with young pineapple plants. They appeared to feed normally on the tender tissue at the base of the leaves and even inserted their eggs in crescent-like slits that they cut in the epidermis. On October 23, I found a pineapple leaf in the field with similar punctures containing fresh eggs. From none of these studies, however, was yellow spot reproduced.

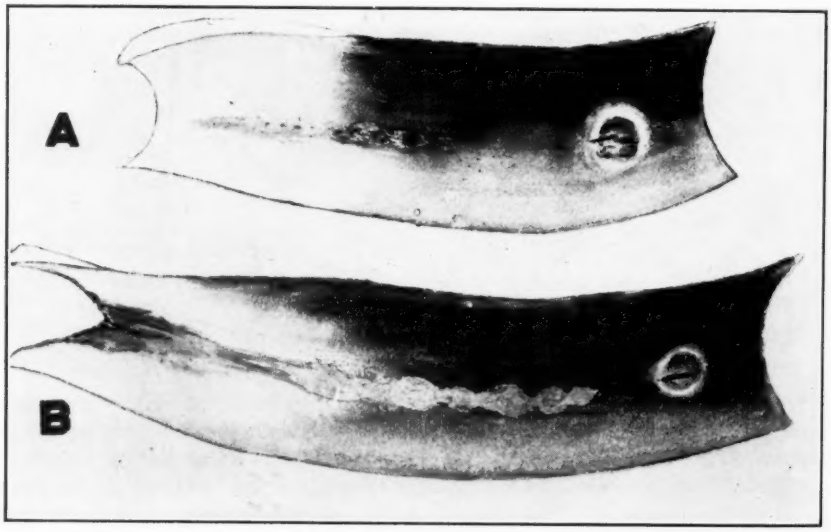

FIG. 8. Two pineapple leaves showing initial yellow spots above. A. An early stage of the disease, with the water-soaked areas just beginning to appear at the base, indicated by shading. B. An advanced stage, showing the characteristic chain of secondary yellow spots, with rot well advanced at the base. Photograph of colored plate by Armena Eller.

Based on the assumption, from analogy, that the vector of yellow spot might be one of the homopterous bugs, suspicion at this time centered upon the three-cornered alfalfa hopper, *Strictocephala festina* (Say). This insect was first reported in the Islands in 1925, so this fact appeared to strengthen the hypothesis that it was the vector and that our pineapple virus possibly came with it. Although this insect had rarely been observed in pineapple fields, it is a strong flier and could easily migrate long distances. The fact that it commonly lived and bred upon Crotalaria and other leguminous plants was also taken into consideration, for many such weeds occur in the sections where pineapples are grown. These hoppers were collected in vast numbers in alfalfa fields and were caged by hundreds with pineapple plants, together with Crotalaria suffering from a mosaic disease. The insects appeared to feed freely upon pineapple leaves, but they failed to survive in the cages. No infections resulted from the feeding punctures, so they, too, had to be rejected.

Microscopic study of the diseased pineapple leaf by Dr. Royal N. Chapman disclosed very tiny, characteristic punctures, usually present in or near the initial spot. The supposition for a time was that these punctures were made by the mouth parts of some sucking insect. Sections cut through them by Dr. M. B. Linford indicated that they were, however, the nidus of an egg of some very tiny insect. Thus the search for the vector became quite sharply defined. The insect evidently was considerably smaller than any of the homopterous bugs known to be present in the Islands. Hence, such bugs were now eliminated.

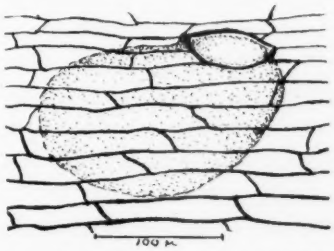

FIG. 9. Sketch of a thrips egg in pineapple leaf, magnified about 175 diameters. The puncture in the epidermis could be seen easily, but to observe the shadowy outline of the egg embedded in the mesophyll required a strong transmitted light.

We used tanglefoot extensively in the infected fields to trap transient insects, but even this method shed little new light on the subject. The same kinds of bugs were captured that had been collected on weeds. There was one addition, a minute Mirid, *Leucopoecila albofasciata* Reuter, which, for a time, became a suspect, chiefly due to the fact that members of the family Miridae are known normally to insert their eggs singly into plant

tissue. Then, again, this insect was so small that there was a possibility that its egg might fit the minute punctures. These bugs fed freely upon pineapple leaves, where they also oviposited. Measurements of the ovipositor, however, forced us to abandon this insect as a suspect, for its puncture was considerably too large. The region on the leaf where the egg was inserted developed a slight water-soaked appearance, with some discoloration, but there was no development of yellow spot.

About this time there first appeared evidence of reproduction of the disease in our cage experiments. Crotalaria plants showing marked mosaic yellowing and infested by aphids were collected in a field where yellow spot was present on pineapples. (I discovered later that the flowers were infested also with thrips.) These plants were potted, together with seedling pineapples, and enclosed in large lantern chimneys, October 7, 1929. November 4, a pair of initial spots began to show about 3 inches from the base on one of the pineapple leaves. As growth continued, the plant passed through all stages of the disease and finally rotted.

Hence it will be seen that the disease required almost a month to incubate before it made its appearance as a yellowing spot on the leaf. A second experimental plant was attacked with the disease after a period of 29 days with similarly infested Crotalaria. Plant lice are well-known vectors in some types of mosaic, but they could not possibly produce the punctures found so regularly in the vicinity of the initial spot.

We next turned our attention to a very tiny Anthrocorid bug, *Triphleps persequens* F. B. White, which preys upon aphids, thrips, and other insects. Great numbers were collected and caged on pineapple plants. They inserted their beaks and fed freely on the white portion of the leaf and also used their sword-like ovipositor to embed an egg here and there in the mesophyll. While these came much nearer to the size required than anything that we had had so far, they were still too large. Furthermore, none of the plants developed any sign of yellow spot.

Seeking a still smaller insect, I finally began an examination of the various species of thrips that occur in and about pineapple fields. One, referred to above in a preceding paragraph, in the flowers of diseased Crotalaria, is omnipresent on many field weeds. I suspected it as being the vector in the two instances cited. This species has a saw-like ovipositor (Fig. 10), with which eggs are inserted singly into the mesophyll of plants. With strong transmitted light the eggs embedded in the stamen tube of Crotalaria flowers were examined, and it was found that the punctures agreed perfectly both in size and shape with those that occur in diseased pineapple leaves. Hence, there appeared to be little question that we were at last on the right lead. Moreover, there was considerable evidence of

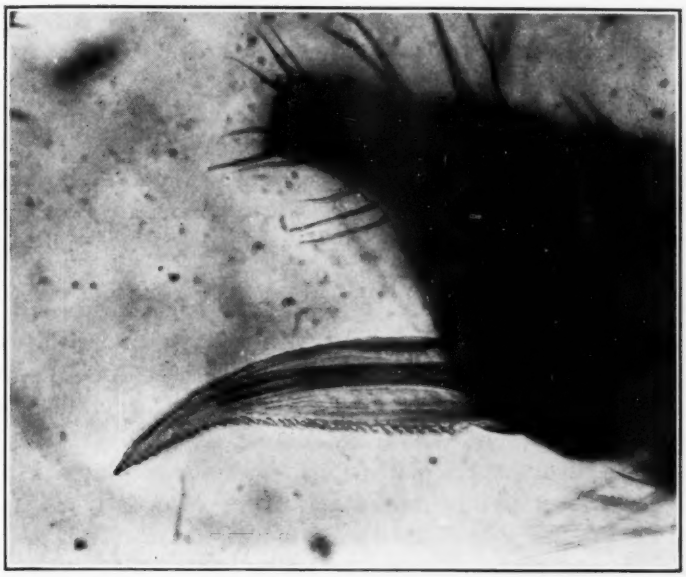

FIG. 10. The end of the abdomen of an onion thrips, *Thrips tabaci* Lind., showing the efficient, serrate ovipositor with which the eggs are inserted into the tissues of the plants. Highly magnified. Photograph by Dr. C. P. Sideris.

thrips feeding-punctures on the leaves of all affected pineapple plants. Infection, undoubtedly, is caused by the mouth parts. Ovipositing in the vicinity of the yellow spot is probably only incidental.

As a preliminary step, we collected thousands of thrips from various weeds and flowers in the affected localities and enclosed them over small seedling pineapple plants. Several species of thrips were concerned, but the onion thrips was remarkably scarce at the time, due to heavy rains. None was observed among those collected. Many of the thrips congregated between the imbricated leaves and evidently fed, for characteristic scars were present in the tender white epidermis. In several instances I located fresh eggs inserted into this same portion of the leaf. One of these eggs was sketched, using the camera lucida with a very strong, transmitted light projected through the leaf from below (Fig. 9). None of these plants developed the disease. After resigning from the Station, I continued the experiment, collecting thrips under more favorable weather conditions. At that time the onion thrips was much in evidence. Two of the pineapple plants used in the experiment soon developed the characteristic yellow spot. This evidence was very encouraging, pointing to thrips as the vector.

Though several species of thrips were included in this experiment, *Thrips tabaci*,[5] which occurs in the affected area on the leaves and in the

[5] This determination verified for me by Dudley Moulton in his letter of Feb. 18, 1930,

flowers of several weeds, became a strong suspect.[6] The recorded host plants of this species (2) include an extensive list of weeds and grasses. It bears, near the end of its abdomen, a strongly serrate ovipositor (Fig. 10), well suited to produce the punctures that we have noted.

As for records of thrips as a vector in the literature, I find nothing conclusive. However, there are several instances where these insects were thought to be responsible for the transfer of mosaic. Boning (1) had mosaic transmission from beets to spinach when only the onion thrips was in evidence. Another instance: Ogilvie (4) reported mosaic transmission on lilies, the only insects present being thrips (onion?) and a mealy bug. The same author (5) states that onion thrips can be controlled by two applications of nicotine sulphate, ½ pint to 50 gallons with 2 pounds of soap added. No results were obtained with nicotine dust. Triphleps, predaceous on these pests, checked them in May.

Several excellent papers dealing with the biology of onion thrips are available. The best of these are in a series by Wardle (6), MacGill (3), and Wardle and Simpson (7). These studies were made on cotton in the United States and Egypt. The observations of the authors that thrips injury was particularly destructive along windward edges with relatively little destruction to leeward fits exactly the situation with yellow spot pineapples. Thrips, being exceedingly small, are commonly distributed by the wind. Their wings are so feather-like that it is impossible for them to make progress even into a light breeze. Again, it was found in the cotton investigation that heavy rains materially cut down thrips injury, since these pests are so poorly protected. This observation, too, just fitted the situation we had during January, 1930. No yellow spot appeared in our fields following the heavy rains. Furthermore, it was observed on cotton that drought and high temperatures of summer slowed down the thrips injury to the lowest ebb. This, too, fits for yellow spot of pineapples. In Egypt, thrips were found to be most prolific during periods of light showers and moderate weather. It is noted that yellow spot has been most destructive under such conditions (Fig. 4, Graphs for 1926, 1928, and 1929).

COMBATING THRIPS ON PINEAPPLES

Control measures, of course, are only tentative at this time. Since yellow spot on pineapples probably comes from weeds affected with mosaic, clean culture is of prime importance. With this crop thrips do their dam-

[6] I have since learned that Dr. M. B. Linford, who took over the investigation, concurrently reproduced the disease and subsequently has established final proof of its relationship to the disease, as reported in the Station's files and in a paper accepted for publication in *Science* as a preliminary report.

age down in the axils of the leaves. To reach them it is necessary to treat only the heart of the plant. Theoretically, the insects could be kept out with some sort of a mechanical plug. Various substances have been tried, including tobacco dust, wheat bran, rice hull, volcanic black sand, etc. A deterrent added to these, composed of an oil emulsion with tobacco extract, increases efficiency. Dusting sulphur, used with success against thrips in California, has good possibilities. The combination of tobacco and sulphur, under the trade name Nico-sulfur, is also being tried. Tobacco extract, used alone, evidently volatilizes too quickly, for it has not shown results.

Weather conditions influence greatly the need for control measures. As indicated in earlier paragraphs, both heavy rains and droughts appear to be fatal to thrips. Natural enemies, too, have an important bearing upon their control. In the United States, where these have been studied, we learn that several species of ladybird beetles and their larvae prey upon thrips. The various species of Triphleps also are well-known predators of these pests. Many other insect enemies, including parasites, have been recorded, but information dealing with them is meager.

In Hawaii we find *Triphleps persequens* omnipresent in flowers or anywhere that thrips congregate. As soon as these bugs become abundant in spring, thrips become scarce. Another important predator, everywhere in pineapple plants, is the Cucujid beetle, *Cryptamorpha dejardinsi* Guer. Both the adults and their larvae live (deep down) in the axils of the leaves, where they prey upon any small insects that come in their way. Without such friends the growing of crops would be quite impossible.

SUMMARY

A mysterious new disease of pineapples appeared in Hawaii during 1926. It usually was evidenced by a breaking-down of the tissues at the base of one or more leaves, the stem soon bending over in the direction of the injured part; the whole plant eventually succumbing. This led to the name side rot. From the subsequent discovery that an initial yellow spot on one or more leaves usually preceded the rotting at the base, the name was changed to yellow-spot disease.

This disease was most troublesome on newly planted tops because of their open structure. Soon it was found to attack these even while attached to the fruits. All attempts to control the disease with sprays proved futile.

Each year the disease increased, and it spread widely in the Islands. During the fairly dry season of 1929 it was more than tenfold what it had been in previous years. At that time it was assumed that it might be a virus disease. It was soon learned that it could not be reproduced by artificial means. Then began a search for the vector among the numerous in-

sects associated with the plants. Finally, with large numbers of the common insects eliminated, the search was narrowed down to one whose characteristics, even before its discovery, were sharply defined by histological studies on the part of other workers on the problem. At the time this paper was written a species of thrips was a strong suspect. I am informed that the final proof of its relationship to the disease has been established by Dr. M. B. Linford and has been accepted for publication as a preliminary report in *Science*.

FORMERLY WITH THE EXPERIMENT STATION,
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HONOLULU, HAWAII.

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BACTERIA ANTIBIOTIC TO USTILAGO ZEAE[1]

R. H. BAMBERG[2]

INTRODUCTION

In the spring of 1928 the writer began an investigation of the factors affecting the infection of corn plants with *Ustilago zeae* (Beck.) Ung. During the course of the field work in the summer of 1928 it became evident that young plants seldom became infected and that many older plants which had been artificially inoculated failed to become infected. Discolored areas often developed near the point of inoculation, when the inoculations were made with hypodermic needles. Cultures were made from these discolored areas, and bacteria were isolated that proved to be decidedly antibiotic to *U. zeae*. Because of the fact that this bacterial culture often seemed to prevent infection, even when susceptible corn plants were inoculated by standard methods with virulent cultures of *U. zeae*, this phase of the problem was further investigated. The results are presented in the following pages.

SOURCES OF CULTURES

Several cultures of bacteria were isolated in the summer of 1928 from corn plants that had been inoculated with *Ustilago zeae* but had failed to develop smut galls. Instead of smut galls, which usually develop on plants inoculated with virulent lines of smut, there were brownish rotted areas on the plants around the inoculation courts. The cultures were obtained from the inner tissues of these rotten areas. Transfers were made with a sterile needle from these diseased tissues to Petri dishes containing 1.5 per cent potato-dextrose-agar. Transfers then were made from resulting colonies to potato-dextrose-agar slants, and these were used as stock cultures. All cultures were grown on potato-dextrose-agar and kept in the laboratory at room temperature unless otherwise stated. Cultures B–1, B–2, B–3, B–4, B–5, and B–6 were obtained in this way. Other cultures of bacteria were obtained from contaminations that appeared in smut colonies that developed from chlamydospores taken directly from smut galls. Still others were procured from contaminations that appeared in stock cultures in the laboratory. Bacterial cultures B–7, B–8, B–9, B–10, B–11, B–12, B–13, B–14 and B–15 were obtained in this way.

[1] Paper No. 963 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer wishes to express his appreciation for the assistance given by Dr. E. C. Stakman, under whose direction these investigations were made. He also wishes to express his indebtedness to Dr. J. J. Christensen for many helpful suggestions and criticisms.

A combination of 4 monosporidial lines of *Ustilago zeae* was used for all inoculations made on corn. Three of these lines were of one sex and one of another, and the combination of these 4 lines had produced heavy infection in previous pathogenicity tests.

Sporidia were obtained by growing the 4 lines of smut on a potato decoction containing 1 per cent malt and 1 per cent dextrose. An abundance of sporidia was produced on this medium. The smut cultures were grown separately until they were to be used. The bacteria were grown in a similar medium. They were mixed with the smut cultures by adding from 5 to 10 cc. of the suspension of bacteria to approximately 50 cc. of the mixture of lines of smut.

Inoculations on corn plants 8 to 10 inches high were made in the greenhouse by hypodermically injecting about ½ to 1 cc. of the suspension of sporidia or of a mixture of sporidia and bacteria into each plant at each of 3 places, namely: (1) at the surface of the ground, (2) about 1 inch above the ground line, and (3) about 1½ to 2½ inches above the ground line. Most of this inoculum remained between the tightly rolled leaves, but some of it was lost by being forced up between the leaves to the outside.

EFFECT OF ANTIBIOTIC BACTERIA ON CULTURES OF USTILAGO ZEAE AND OTHER SMUTS

Colonies of *Ustilago zeae* in test-tubes and in Erlenmeyer flasks were inoculated, by transferring a small amount of bacteria with a sterile needle to a point on the margin of the colony, with bacterial cultures B–1, B–2, B–3, B–4, B–5, and B–6. Colonies about 1 inch in diameter were completely surrounded by the bacterial growth in from 3 to 5 days, and the further growth of the fungus was stopped. Before inoculation the surface of smut colonies was rather dry but became wet and slimy as the bacteria grew and spread. The action on colonies of smut appeared to be very similar for all 6 of these bacterial cultures. For more intensive study only 1 culture, B–1, from these isolations was used.[3]

Colonies of *Ustilago zeae* that had been growing on potato-dextrose-agar in 250 cc. flasks for 9 days were inoculated with culture B–1. In 6 or 7 days the cultures of smut were changed from compact colonies that could all be lifted from the medium with an inoculating needle to slimy colonies of which only a small portion could be lifted with a needle. The colonies of smut were changed to slimy masses with very little or no fungus growth left. The appearance of the colonies is shown in figure 1. These experiments were repeated many times and the results were in general always the same.

[3] The purity of this culture was not established, but it was turned over to Dr. Delia Johnson for further work.

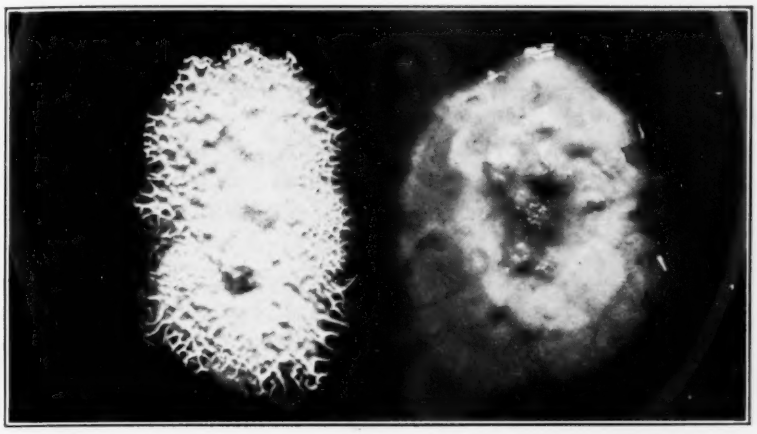

FIG. 1. Seventeen-day-old cultures of *Ustilago zeae* growing on potato-dextrose agar. The colony on the right, when 9 days old, was inoculated with bacterial culture B–1.

Colonies of smut in test-tubes appeared to be affected the same as those in flasks. Tough cultures of the smut fungus seemed to be almost completely dissolved. When examined microscopically only a very few sporidia and segments of mycelium were left in the colonies of *Ustilago zeae* that had been inoculated with B–1, while in pure cultures of smut of the same age there was almost a solid mass of sporidia and mycelium.

Colonies of *Ustilago zeae*, approximately 1 to 1½ inches long, on agar slants were inoculated on the lower margin with B–1 and 4 colonies incubated at each of the temperatures 10, 15, 20, 25, and 30° C. The bacteria were able to destroy the smut colonies at all these temperatures but the lytic effect was most rapid at 25° and 30° C. Figure 2 shows the average rate of advance of the bacteria through the fungus colonies.

Colonies of *Ustilago zeae* on agar slants were also inoculated with bacterial cultures B–7, B–8, B–9, B–10, B–11, B–12, B–13, B–14, and B–15 to determine the effect of these other bacteria on the growth of smut colonies. Four of these bacteria greatly retarded or prevented further development of the colonies of smut, but none caused such complete dissolution as did B–1 and the other cultures from the same source. Colonies of *U. zeae* inoculated with the other 5 bacteria continued to grow normally and increase in size, although culture B–11 seemed temporarily to delay the development of a young culture of smut, which later developed normally.

These results indicate that some bacteria completely prevent growth of *Ustilago zeae* on solid culture media and may even destroy fully developed colonies, while other bacteria have no appreciable effect.

Cultures of *Ustilago zeae* 6 days old, growing in malt-dextrose-potato decoction, were inoculated with B–1. At the time of inoculation, mounts

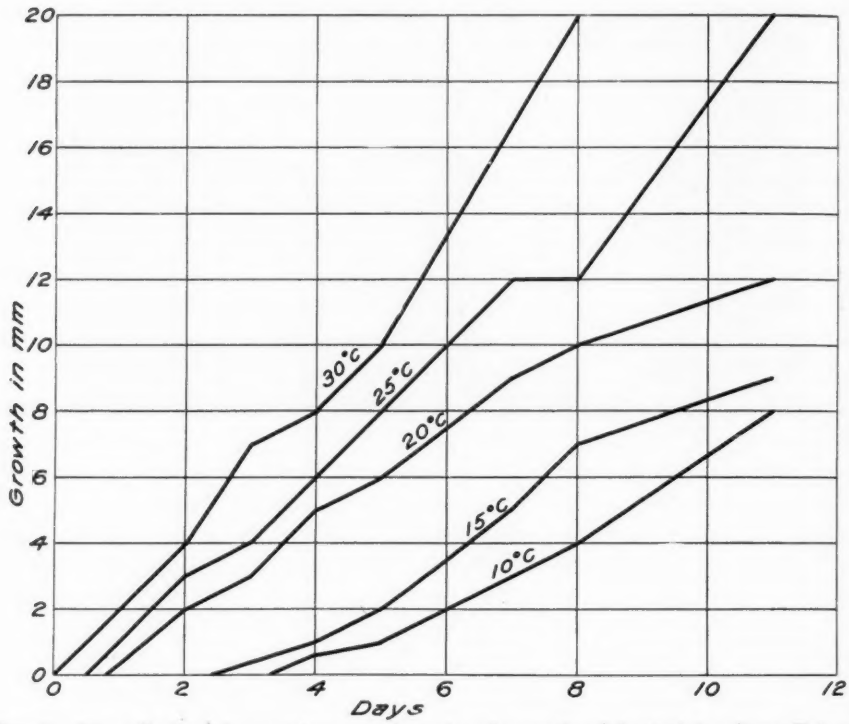

FIG. 2. The effect of temperature on the rate of growth of bacterial culture B-1 on colonies of *Ustilago zeae*, as measured by the length of the bacterial colonies at different intervals of time.

of the sporidial suspension were examined under the microscope. A large number of sporidia were found in all the flasks. Ten days later the flasks inoculated with B–1 contained a much smaller number of sporidia of *U. zeae* than did the flasks which contained the pure cultures of *U. zeae*. These experiments were repeated a number of times, with the same general results. There might have been two reasons for the smaller number of sporidia in the flasks inoculated with the bacteria: (1) direct action of the bacteria on the sporidia, resulting in their disintegration, and (2) production of an environment unfavorable to the development of the smut fungus.

In order to determine whether the bacteria were directly responsible for the disintegration of the sporidia a large number of hanging drops of a sporidial suspension of *Ustilago zeae* were set up in van Tieghem cells and inoculated with B–1. Other drops in cells on the same slides were set up and left uninoculated as checks. The sporidia in these hanging drops were observed through the microscope at frequent intervals. After about 24 hours the number of sporidia in the drops not inoculated with the bacteria

was considerably greater than in the inoculated drops. After about 36 hours a mass of new sporidial growth was clearly visible with the naked eye on the surface of drops not inoculated with B–1, while no such growth could be seen on any of the drops inoculated with B–1. Instead of an increase in the number of sporidia in the hanging drops inoculated with B–1 there was a rather rapid decrease in most cases. With this decrease in number of sporidia what appeared to be particles of disintegrated sporidia could be seen in some of the drops that had been inoculated with B–1. These particles were irregular bodies of no definite shape, usually larger than the bacteria but smaller than the sporidia. Similar particles could not be seen in any of the drops not inoculated with B–1.

Since the bacteria had a marked antibiotic effect on cultures of corn smut, an attempt was made to determine the effect of one of these cultures on some other smuts. Five colonies of *Ustilago avenae* and 2 of *U. levis* in test-tubes were inoculated with B–1. Seven days after inoculation all growth apparently had been stopped. In 4 weeks the tough mycelial colonies were changed to a rather slimy mass and the dissolution seemed almost as complete as in the case of *U. zeae*.

The inoculations of *Ustilago avenae* and *U. levis* were repeated, using 3-day-old colonies of both smuts. Four cultures each of 2 forms of *U. avenae* and 1 form of *U. levis* were inoculated with B–1, and 2 cultures of each were used as controls. The colonies used as controls continued to develop rapidly, but those inoculated with B–1 were reduced to a slimy mass in 5 days.

Inoculations of B–1 on *Sorosporium reilianum* (Kühn) McAlp. and *Tilletia tritici* (Bjerk.) Wint. indicate that the bacteria are destructive to colonies of *S. reilianum* and very injurious to the growth of *T. tritici*. The colonies of *S. reilianum* seem to be dissolved fairly readily. Colonies of *T. tritici* were prevented from further growth, but dissolution of the colony took place very slowly. This may have been because the colonies were very old and tough before they were inoculated with B–1.

The effect of the bacteria on infection of corn with Ustilago zeae. Laboratory investigations indicated that the growth of *Ustilago zeae* in pure culture was greatly retarded or prevented by certain cultures of bacteria and that the sporidia were disintegrated by them. Therefore, experiments were made in the greenhouse to determine the possible effect of the bacteria on the infection of corn plants by *U. zeae*. Sporidial suspensions of 4 monosporidial lines of *U. zeae* were grown separately in flasks containing malt-dextrose-potato decoction. These monosporidial lines were mixed together by straining into a large flask through cheesecloth to remove any material that would not pass through a hypodermic needle. This mixture

of smut lines was then divided into 2 equal parts and a suspension of B–1 in the same kind of medium added to one half at the rate of 1 part of bacterial suspension to 2 parts of smut inoculum. Another type of inoculum used was a mixture containing the same 4 monosporidial lines of smut and bacterial culture B–11. Treatments with distilled water were made as controls.

On November 3, 1928, a number of plants of Golden Bantam corn, 8 to 10 inches high, growing in beds of soil in the greenhouse, were inoculated with each of the different kinds of inoculum given in table 1. Notes, as summarized in table 1, were taken on November 23.

TABLE 1.—*The effect of bacterial cultures B–1 and B–11 on the development of smut in corn plants inoculated with Ustilago zeae*

Material injected	Number of plants		Percentage of infection
	Inoculated	Infected	
Distilled water	95	0	0
Four monosporidial lines of *U. zeae*	104	75	72.1
Four monosporidial lines of *U. zeae* plus B–11[a]	103	74	71.8
Four monosporidial lines of *U. zeae* plus B–1	102	8	7.8

[a] In this case bacterial culture No. B–11 was grown in association with 1 monosporidial line of the corn smut.

Two plants inoculated with *Ustilago zeae* and B–1 were decayed in the bud, very much like those from which the bacteria were first isolated. Several isolations of bacteria were made from these 2 plants. These appeared to be the same as the original cultures used as inoculum. Colonies of *U. zeae* in test-tubes inoculated with these reisolated bacteria were destroyed.

Further inoculations were made into Gehu Flint corn on February 18, 1929, using only the 4 monosporidial lines of *Ustilago zeae* and the 4 lines plus B–1. Notes were taken March 3. The results, given in table 2, are similar to those for the previous inoculations.

TABLE 2.—*The effect of bacterial culture B–1 on the development of smut in corn plants inoculated with Ustilago zeae*

Inoculum	Number of plants		Percentage of infection
	Inoculated	Infected	
Four monosporidial lines of *U. zeae*	55	29	52.7
Four monosporidial lines of *U. zeae* plus B–1	105	8	7.6

Tables 1 and 2 show rather conclusively that, when B–1 is mixed with the smut at the time of inoculation, the percentage of infection by *Ustilago zeae* is greatly reduced. Table 1 further shows that certain other bacteria, such as culture B–11, did not reduce the amount of infection.

In order to determine the effect of the bacteria on the development of the smut when injected into the plant at times other than that of inoculation with *Ustilago zeae*, a series of inoculations with bacteria was made before and after the time of injection with the suspension of sporidia. The first inoculations with bacteria were made 5 days before those with the smut. When B–1 and *U. zeae* were injected the same day the suspension of bacteria and sporidia were mixed together before injection. Readings on infection percentages were made April 7, 1929. The results of the inoculations are given in table 3.

There were 3 rather distinct types of infection and the percentage of each caused by the different kinds of inoculum is given separately. In some plants there was either chlorosis or red coloration of the leaves without the formation of definite galls or warty thickenings on the leaves. Plants having this type of infection were classed under "chlorosis or anthocyanin coloring." In some there were wart-like thickenings on the leaves or sheaths. Rounded galls with spores had not fully formed. These were classed as "incipient galls." Under "large galls" were placed those plants that had well-formed galls containing spores.

These results indicate that the effect of B–1 on the development of *Ustilago zeae* in the host is more pronounced when injected into the plant at the same time as the smut but that a considerable influence of the bacteria is retained when they are injected for at least 3 days prior to inoculation with smut. Apparently, the amount of infection is significantly reduced when the plants are injected with B–1 1 and 3 days after inoculation with smut. This is especially true if one considers the severity of infection as indicated by the size of galls and extent of chlorosis.

Even after smut galls have attained considerable size they may be disintegrated by the action of the bacteria and prevented from forming spores. Thirty or more galls $\frac{1}{4}$ inch to $\frac{3}{4}$ inch in diameter were inoculated on the plants by injecting a small amount of bacterial suspension into them with a hypodermic needle. Almost all the galls dried up after 2 weeks, without producing chlamydospores. Forty other galls, about the same size as those inoculated with B–1 on the plants, were placed in Petri dishes with portions of the host plants attached. Two large galls were put in each Petri dish. One was inoculated with B–1 by pricking with a needle which had been dipped into a colony of the bacteria; the other was pricked with a sterile needle. In 24 hours a slimy exudate came from 2 of the inoculated

TABLE 3.—*The effect of inoculating corn plants with Ustilago zeae at different periods after the plants had been inoculated with bacterial culture B–1 and at different periods preceding the bacterial inoculations*

Inoculations		Plants inoculated	Total infections	Plants with		
Inoculum	Date			Chlorosis or anthocyanin coloring	Incipient galls	Large galls
		No.	Per cent	Per cent	Per cent	Per cent
B–1	March 18, 1929					
U. zeae	March 23, 1929	101	79.2	22.8	28.7	27.8
B–1 with						
U. zeae	March 23, 1929	106	56.6	35.8	12.2	8.4
B–1 with						
U. zeae	March 23, 1929	102	48.0	40.2	6.8	.9
U. zeae	March 23, 1929	118	90.6	33.9	27.1	29.6
U. zeae	March 23, 1929	103	81.5	20.4	21.3	39.8
U. zeae	March 23, 1929					
B–1	March 24, 1929	122	73.7	37.7	18.8	17.2
U. zeae	March 23, 1929					
B–1	March 26, 1929	116	68.1	31.9	22.4	13.8
U. zeae	March 23, 1929					
B–1	March 28, 1929	113	77.0	30.9	26.5	19.4
U. zeae	March 23, 1929					
B–1	March 30, 1929	106	83.9	23.5	26.4	34.0

galls. In 4 days all of the galls inoculated with B–1 were almost completely disintegrated.

These investigations with *Ustilago zeae* in culture and in the host plant have shown rather conclusively that certain cultures of bacteria, and 1 culture in particular, designated B–1 for convenience, in some way have a deleterious effect on the development of the smut both in cultures and in the host. An attempt was made to determine whether it was the direct effect of the bacteria on the smut fungus or the effect of some by-product of the metabolism of the bacteria. Two cultures of the bacterium, B–1, grown on malt-dextrose-potato decoction, 1 for 4 and the other for 7 days, were filtered through sterile Berkefeld filters (medium). Colonies of *U. zeae* in test tubes to which a loop of the filtrate was transferred continued to grow apparently the same as control colonies to which no filtrate had been added. No growth appeared on agar slants to which loops of the filtrate were transferred. Part of the filtrate was poured into a mixture of

sporidia of 4 lines of *U. zeae* at approximately the ratio of 1 part of the filtrate to 4 parts of the sporidial suspension, and the whole allowed to stand 24 hours. Table 4 gives the results of the inoculation of plants with this mixture as compared with inoculation with the same lines mixed with B–1. Apparently, there was nothing in the filtrate which inhibited or retarded the development of *U. zeae*.

Antibiosis between microorganisms has been observed by many workers. A general review of the literature dealing with the phenomenon, given by Buchanan and Fulmer,[4] indicates that the action is quite variable. This aspect of the problem will be considered in more detail in a paper by Delia Johnson.

TABLE 4.—*The ineffectiveness of a sterile filtrate from B–1 on the development of U. zeae*

Inoculum	Plants inoculated	Infections
	No.	Per cent
U. zeae + filtrate from B–1	37	72.9
U. zeae + B–1	33	0

DISCUSSION AND CONCLUSIONS

Certain cultures of bacteria were found to inhibit the development of *Ustilago zeae*. Experiments proved that these bacteria were capable of preventing the multiplication of sporidia of *U. zeae* and of destroying colonies of *U. zeae* and of some other smuts already formed in culture. The wide-spread distribution of such bacteria may serve as an important check on the multiplication of inoculum in the soil.

A culture of bacteria which prevented the growth of *Ustilago zeae* in culture also greatly reduced the percentage of smut infection when injected into the host along with the sporidial suspension. In one of the pathogenicity tests the percentage of infected plants was 72.1, where a combination of 4 monosporidial lines of smut was used as inoculum, in contrast to 7.8 where the same combination of smut lines mixed with the bacteria was used. The fact that certain bacteria do prevent smut infection of corn plants to a great extent may account for many of the irregularities observed in the percentage of infection resulting from artificial inoculations. It may also account for many of the variations in the amount of smut oc-

[4] Buchanan, R. E., and Ellis I. Fulmer. Physiology and biochemistry of bacteria. Vol. III, 575 pp. Williams and Wilkins Co., Baltimore. 1930.

curring under natural conditions in different fields in the same year and in the same field in different years.

The destructive action of such bacteria as were found to destroy smut galls already formed on corn plants may account for the disappearance of smut on corn plants in the field. The presence of such bacteria also may account for the appearance of abortive galls often observed. Corn smut, attacked early in its development on the host, may fail to develop normally.

SUMMARY

1. Ten cultures of bacteria were found to have a deleterious effect upon the development of *Ustilago zeae* in culture, while 5 others had no observable like effect.

2. Sporidia of *U. zeae* apparently failed to multiply in the presence of a culture of bacteria isolated from corn plants which failed to produce galls when inoculated with *U. zeae*.

3. Evidence of disintegration of sporidia by the bacteria was obtained.

4. A combination of monosporidial lines of *U. zeae* was made less virulent in its attack on the host by the association with these bacteria.

5. The injurious effect of the bacteria on the development of the smut in the host persisted when injected into the corn plant at least 3 days before inoculation with *U. zeae*. Apparently, the bacteria were able to live in or on the corn plant at least that long.

6. The virulence of the smut seemed to be significantly reduced even when the bacteria were injected into the plants as much as 3 days after inoculations with smut.

7. Smut galls were disintegrated and spore formation was prevented even after distinct galls more than $\frac{1}{2}$ inch in diameter had been formed.

8. The destructive action seems to be directly associated with the presence of the bacteria, as the filtrate from cultures of the bacteria had no appreciable effect on colonies of smut in culture or on the development of smut galls in inoculated plants.

FIELD STUDIES ON THE RING–SPOT DISEASE OF BURLEY TOBACCO IN WASHINGTON COUNTY, VIRGINIA[1]

S. B. FENNE[2]

INTRODUCTION

Tobacco ring spot is a virus disease that is becoming more and more prevalent in the State every year. In 1917, when the disease was first observed by Fromme and Wingard, it was considered of minor importance. Infection at that time was observed on only an occasional plant in a field and the crop loss was negligible. The amount of infection, however, has increased from year to year until now it is not uncommon to find fields in which 90 per cent of the plants are infected.

The writer studied this disease under field conditions in 10 counties of the State during the summer of 1927 and made a special study of it on Burley tobacco in Washington County during the seasons of 1928, 1929, and 1930. The disease was first described and illustrated as ring spot by Fromme and Wingard.[3]

Fromme, Wingard, and Priode[4] recorded ring spot in 11 counties in Virginia. They proved that the ring-spot disease was infectious and concluded that it should be classed with the virus diseases. Wingard[5] reported that the ring-spot virus had a very wide host range, although it was very specific in its infective properties, since many plants that were inoculated failed to develop the disease. He was the first to find natural ring-spot infection on any plant other than tobacco. Repeated inoculations on tobacco, with expressed juice from sweet clover, produced typical ring-spot symptoms.

MATERIALS AND METHODS

To determine if certain weeds were harboring the ring-spot virus, suspected plants growing in or around the plant beds and fields were sent to Dr. Wingard at the Virginia Agricultural Experiment Station for inoculation tests on healthy tobacco plants.

[1] Condensed from a thesis presented to the Department of Botany and Plant Pathology of the Virginia Polytechnic Institute in partial fulfillment of the requirement for the degree of Master of Science.

[2] The writer is particularly grateful to Dr. S. A. Wingard, under whose direction this work was done, for correcting the manuscript and for invaluable suggestions, advice, and encouragement.

[3] Fromme, F. D., and S. A. Wingard. Blackfire or angular leaf-spot of tobacco. Va. Agr. Exp. Sta. Tech. Bul. 25. 1922.

[4] Fromme, F. D., S. A. Wingard, and C. N. Priode. Ring-spot of tobacco, an infectious disease of unknown cause. Phytopath. 17: 321–328. 1927.

[5] Wingard, S. A. Hosts and symptoms of ring-spot, a virus disease of plants. Jour. Agr. Res. 37: 127–153. 1928.

The insects studied were secured from the writer's vegetable garden and placed on ring-spot-infected tobacco plants in cages and left for different periods of time. They were then transferred to caged healthy tobacco plants. Tobacco flea beetles, *Epitrix parvula* Fabr., were secured from tobacco plants. Potato or cucumber flea beetles, *Epitrix cucumeris* Harr., and leaf hoppers, *Empoasca fabae* Harr., were secured from potatoes. Aphis, *Macrosiphum solanifolii* Ashm., were secured from potatoes and clover. The tobacco worm, *Phlegethontius quinquemaculata* Haworth, and the firefly, *Photinus scintillans* Say, were found on tobacco plants. The insect catcher described by Kunkel[6] was used.

The extent of injury to affected plants was determined by counting and measuring the leaves on diseased and healthy plants at topping time. The quality of the leaf also was taken into consideration. Numerous inspections and field counts of affected plants gave the number of plants diseased. The total loss to the county was estimated by multiplying the average extent of injury to the individual plant by the total percentage of affected plants. Judy's Pride, Kelley's, and Lockwood varieties of tobacco were used in all experiments conducted in Washington County.

EXPERIMENTAL RESULTS

Since ring spot is becoming of such great economic importance and so little is known regarding it, experiments were undertaken in an effort to determine the source of inoculum, means of dissemination of inoculum, the rate of spread of disease in the field, the extent of injury to affected plants, the percentage of plants affected, and the loss to the county.

SOURCE OF INOCULUM

Four possible sources of inoculum suggested themselves: (1) seed, (2) insect vectors, (3) weed hosts, and (4) soil.

Seed: In studying the possibility of the transmission of ring-spot virus by tobacco seed, plant beds in 6 counties in Virginia were inspected in 1927. In only 3 plant beds in 1 county was ring spot found, and this was late in the season, after all transplanting had been completed. Therefore, it is safe to assume that the infection did not necessarily originate within the plant bed.

In 1928, 1929, and 1930, close observation was kept on seed beds in Washington County. They were visited at short intervals until transplanting time. In 1928 and 1929, no ring-spot infection was found in the plant beds. In 1930, however, plant-bed infection was observed in 10 per cent of the beds inspected in Washington County. This observation was surprising, since plant-bed infection had been regarded as very rare up to that time. During the same season, however, plant-bed infection was re-

[6] Kunkel, L. O. Studies on aster yellows. Amer. Jour. Bot. 13: 646–705. 1926.

ported by Godkin[7] in one other county in Virginia and by Valleau[8] in Kentucky.

This opens up a new problem formerly thought settled. Did the plant-bed infection arise from infected seed, or were the young plants inoculated by some insect that carried the inoculum from near-by weed hosts, which were in all cases very abundant?

Insect studies: Because of the similarity between ring spot and certain insect-borne virus diseases, it was thought advisable to study certain insects as possible vectors of the ring-spot virus. The tobacco flea beetle attacks tobacco plants both in the plant bed and in the field. For this reason it was looked upon as a possible disseminator of the ring-spot virus. On June 11, 75 flea beetles were collected from healthy tobacco plants and placed in cages on ring-spot-affected tobacco plants. On June 22, 15 of these beetles were transferred to 2 healthy tobacco plants in an insect-proof cage. On June 24, 30 more of these beetles were removed and placed on healthy tobacco plants in insect-proof cages. In this case 15 beetles were placed on each of 2 plants in separate cages. On July 6, the beetles were alive and thrifty, but no ring-spot infection had developed. On August 2, the plants were normal and free from ring spot.

On July 4, 15 flea beetles were transferred from ring-spot-infected tobacco plants in the field to a healthy tobacco plant in an insect-proof cage. This plant was examined on August 24 and found to be normal in appearance. On July 5, 15 flea beetles that had fed on diseased tobacco plants for 25 days were transferred to a caged healthy tobacco plant. On August 24, no ring spot had developed. These experiments seem to show that the ring-spot virus is not transmitted by tobacco flea beetles.

On June 11, 75 potato flea beetles were collected from potato plants and placed in an insect-proof cage on ring-spot-infected tobacco plants. On examination, 8 days later, all of these beetles were dead.

On June 19, 50 adult and nymph aphids, *Macrosiphum solanifolii,* were placed on diseased tobacco plants in an insect-proof cage. Seven days later all aphids were dead. Apparently, tobacco is not a favorable host for these aphids.

On June 11, 5 tobacco worms were placed in a cage on an infected plant. On July 4, these worms were transferred to a healthy caged plant. On August 24, no ring spot had developed.

On June 24, 50 leaf hoppers were placed on diseased tobacco plants. On July 4, 10 days later, all leaf hoppers were dead. On July 4, 15 leaf

[7] Godkin, James. Tobacco plant bed survey in Virginia. U. S. Dept. Agr., Bur. Pl. Indus. Plant Disease Reporter **14**: 121. 1930.

[8] Valleau, W. D. Tobacco seed beds in Kentucky. U.S. Dept. Agr., Bur. Pl. Indus. Plant Disease Reporter **14**: 113. 1930.

hoppers were transferred from potatoes in the field to a healthy tobacco plant in a cage. On August 2, these leaf hoppers also were dead and no infection resulted.

It seems that some strong flying insect might be responsible for disseminating ring spot since infection was first observed in scattered parts of the fields with apparently no relation to earlier affected plants. The only strong flying insects observed on tobacco were the common fireflies, but in my cage experiments they died after a few days' confinement.

Weed hosts: Many perennial weeds have been found susceptible to the ring-spot disease and many of them, such as the horse nettle and pokeweed, are common in and around tobacco fields. In order to determine whether such plants harbored the virus, suspected specimens were sent to Dr. Wingard to be used as inoculum on healthy tobacco plants. Sweet clover, *Melilotus alba* Desr., on several occasions, produced typical ring-spot symptoms when inoculated on healthy tobacco plants. In July, 1929, the writer sent Dr. Wingard several suspected weeds in which were included stick weed or yellow crown beard, *Verbesina alternifolia* Britton. Healthy tobacco plants were inoculated with the expressed juice from this plant. Nine days later very distinct ring-spot infection was found on the inoculated plants. The stick weed was slightly yellowish with the upper leaves distorted, and rosette-like. No unusual spotting on the leaves was noticeable. Stick weed has since been found by the writer to be a common carrier of the ring-spot virus.

Other plants sent to Dr. Wingard were: prickly lettuce, *Lactuca virosa* L.; broad leaf plantain, *Plantago major* L.; burdock, *Arctium lappa* L.; pokeweed, *Phytolacca decandra* L.; milkweed, *Asclepias* sp.; European bittersweet, *Solanum dulcamara* L.; smart weed, *Polygonum* sp.; lamb's-quarters, *Chenopodium album* L.; broad-leaf dock, *Rumex obtusifolius* L.; button weed, *Malva rotundifolia* L.; Jimson weed, *Datura stramonium* L.; red clover, *Trifolium pratense* L.; mammoth clover, *Trifolium medium* L.; alsike clover, *Trifolium hybridum* L.; and white clover, *Trifolium repens* L.

These plants were sent in at different periods of the year, and some species at several different times. Suspected plants, not only from Washington County but also from Halifax, Amherst, Nelson, Appomattox, Pittsylvania, Mecklenburg, Charlotte, Brunswick, Dinwiddie, and Montgomery counties were used. Negative results were obtained in every case in these tests.

Soil: An experiment was conducted on a large scale on one farm to determine whether the plant-bed soil could transmit the disease. Five hundred square yards of plant-bed soil were thoroughly steam sterilized. As

a result, the plant beds were very clean and free from weeds. The plants were very thrifty and vigorous. Nevertheless, 30 per cent of the plants in fields set from this plant bed developed ring-spot infection. This seems to prove conclusively that the ring-spot infection in this case did not originate in the plant-bed soil.

RATE OF SPREAD IN THE FIELD

After tobacco is set in the field it has been noticed that in some instances a very rapid spread of ring spot occurs. In order to study the rate of spread in the field, inoculations were made in 7 fields and observations made on the rate of spread of infection, at 10-day intervals, through the growing season.

In each case a single tobacco plant was inoculated with the virus, typical ring-spot lesions appeared in every case on the inoculated plants, and the infection became systemic, with all leaves showing symptoms of the disease.

In some cases ring spot was observed in various parts of the field the following day; but, in other cases, it was not observed until 20 days later. In some fields it was found in close proximity to the inoculated plant and in other cases the infection was first noted in remote parts of the field. Affected plants were observed in a very irregular order with apparently no relation to the plant originally inoculated. It, therefore, seems that the infection came from some other source, such as weed hosts. Various kinds of weeds were present in great abundance, surrounding every field in which a spread of ring-spot infection was noted.

PERCENTAGE OF PLANTS AFFECTED

One hundred and seventy-one fields in 10 counties and 49 plant beds in 6 counties were inspected in 1927. An average of 2.5 per cent ring-spot infection was found in the 171 fields observed. In 1 field in Brunswick County ring spot occurred on 75 per cent of the plants.

Fifty representative tobacco fields were examined in 1928 in Washington County. Ring spot was found in 46 of these. An average of 3 per cent infection was recorded for that year.

One hundred tobacco fields in different parts of the county were inspected in 1929. Only 2 small fields were found that did not contain ring spot. Many fields were found with from 50 to 90 per cent infection, but the majority contained from 2 to 12 per cent, with an average of 7.6 per cent. The highest percentage of infection was found in the middle of the growing season, when the plants were about a foot high. At topping time the ring-spot symptoms had been masked on about 50 per cent of the affected plants in most fields.

EXTENT OF INJURY TO THE AFFECTED PLANTS

It is very important to know the extent of injury to affected plants by ring spot; therefore, the leaves on 20 healthy and 20 diseased plants were measured at topping time. The number of leaves, the dimensions of the leaves, the extent of tissue injury, and the quality of the leaves on each plant were determined. The results of this study are shown in table 1.

TABLE 1.—*Average size of leaves on healthy and diseased plants*

Healthy plants		Diseased plants	
Plant No.	Average leaf dimensions in inches	Plant No.	Average leaf dimensions in inches
1	27 × 13	1	19 × 8
2	27 × 14	2	20 × 9
3	23 × 9	3	17 × 9
4	27 × 11	4	25 × 12
5	27 × 13	5	28 × 11
6	27 × 12	6	23 × 11
7	25 × 12	7	23 × 10
8	22 × 11	8	19 × 7
9	28 × 13	9	26 × 12
10	24 × 10	10	24 × 10
11	21 × 12	11	23 × 8
12	26 × 11	12	24 × 10
13	28 × 13	13	25 × 11
14	27 × 14	14	20 × 8
15	23 × 11	15	21 × 9
16	25 × 12	16	25 × 8
17	23 × 12	17	15 × 6
18	26 × 11	18	17 × 8
19	24 × 10	19	17 × 6
20	27 × 12	20	20 × 7
Average—	25 × 12	Average—	21.5 × 9

A study of the data tabulated above shows that the leaves of the diseased plants are 15 per cent shorter and 25 per cent narrower than those of the healthy plants. From these data it is estimated that ring spot caused 33 per cent injury to the affected plants in the reduction of the size of the leaf. In addition to the loss in size, the average number of leaves per plant was 2.25 less for the affected ones. The healthy plants averaged 14.75 leaves per plant and the diseased ones averaged only 12.5 per plant. This shows a 15 per cent reduction in the number of leaves per plant. Since ring spot caused 15 per cent reduction in the number of leaves per plant

and also 33 per cent reduction in the size of the leaves, there was a total loss of 43 per cent per plant caused by this disease. There was also considerable tissue injury to the leaves on the affected plants.

TOTAL LOSS FOR WASHINGTON COUNTY IN 1929

The total loss for Washington County was found by multiplying the average extent of injury to the plant by the percentage of plants affected. According to the records, 7.6 per cent of all the plants in the county were affected with ring spot, and the value of the affected plants was reduced 43 per cent. The total loss for the county, therefore, was 43 per cent of 7.6, or 3.26 per cent of the entire crop.

It is estimated that in 1929 2,500 acres of tobacco were grown in Washington County and that the average production was 1,050 pounds per acre. The average price paid for Burley tobacco on the Abingdon market was 32 cents per pound. Therefore, the total crop produced was 2,652,000 pounds and the loss due to ring spot about 85,575 pounds. This makes a loss of $27,384.00 to the farmers of Washington County as a result of the ring-spot disease, in 1929.

DISCUSSION OF RESULTS

The writer's observations in 11 of the leading tobacco counties of Virginia, representing all types of tobacco grown—Burley, flue-cured, fire-cured, and sun-cured—lead him to believe that the ring-spot virus is not any more virulent for one type or variety of tobacco than it is for another but that all types are about equally susceptible. It has been shown by Wingard[9] that the ring-spot virus in expressed juice does not remain virulent for more than 24 hours at ordinary temperatures. It does not seem possible, therefore, for the virus to be carried over in the plant-bed soil or old tobacco or other plant refuse.

As reported above, various plants and weeds were closely observed in an attempt to determine which were capable of harboring the ring-spot virus. Twenty-five different species of plants were sent to the State Agricultural Experiment Station for inoculation tests with tobacco. Sweet clover and stick weed were the only plants found to be naturally infected with the ring-spot virus.

It seems that some insect must be responsible for transmitting the ring-spot virus from affected weed hosts to tobacco; however, results were not obtained to support this hypothesis. Further study along this line under varying conditions is urgently needed.

The masking of the symptoms of this disease is shown to a very striking degree. Fields were observed in the middle of the growing season to

[9] Loc. cit.: See footnote 5.

be badly affected with ring spot and, in one case, as much as 90 per cent infection was found earlier in the season. Later in the season, about topping time, the symptoms became masked to such a degree that it was difficult to locate them and, finally, many disappeared altogether. The field that showed 90 per cent infection on July 3 showed only 35 per cent infection on August 3. In another field, where counts were made on July 3, 75 per cent infection was noted. On August 3, the infection had become masked to such an extent that only 30 per cent of the plants showed symptoms of ring spot. A similar change was seen in practically every case. The masking of the ring-spot symptoms is very interesting. It occurs both in the field and under greenhouse conditions.

The extent of injury to plants was rather difficult to determine because, even though the plant may have had the disease and shown typical markings early in the season, at topping time and later, when these studies were made, the symptoms in many cases had completely disappeared. It could not be definitely determined whether certain plants were dwarfed as a result of ring spot or because of some nutritional disturbance. However, measurements were obtained which give a fairly accurate idea of the injury caused by the disease. There may be considerable injury not noticeable in the weight and quality of the leaf; and, consequently, the injury due to stunting may be greater than the writer's studies actually indicate.

For 2 consecutive years the sales of ring-spot-affected tobacco have been closely observed on the warehouse floors. Due to the masking of symptoms previous to cutting, it was very difficult to find any evidence of the ring-spot disease in the cured leaves, known to be severely affected during the growing season. There apparently was no reduction in the price paid for such affected tobacco, although there undoubtedly was a considerable reduction in the weight of leaves. Therefore, the total receipts for the crop were considerably less. If the tobacco had been sold in the field before cutting, the buyer most probably would have cut the price on the affected tobacco to a considerable extent, owing to the very conspicuous nature of the ring-spot symptoms.

SUMMARY

1. Steam sterilization of tobacco-plant beds did not prevent ring-spot infection in the fields planted from these beds.

2. Negative results were obtained in all cases in attempts to transmit ring-spot virus by means of tobacco flea beetle, cucumber flea beetle, leaf hopper, aphis, firefly, and the tobacco horn worm.

3. Stick weed, *Verbesina alternifolia*, and sweet clover, *Melilotus alba*, were found naturally infected with ring spot. Infection was readily obtained on tobacco with the expressed juice from these plants.

4. Twenty-five other species of weeds were tested for ring spot, with negative results.

5. The percentage of ring-spot infection in 10 counties in Virginia in 1927 was 2.5 per cent. In Washington County, in 1928, it was 3 per cent and, in 1929, 7.6 per cent.

6. There was an average injury of 43 per cent to the affected plants.

7. It is estimated that ring spot caused a total loss of $27,384.00 in Washington County, in 1929.

THE RELATION OF CANKER TREATMENT TO FIRE-BLIGHT CONTROL

J. A. McCLINTOCK

It has long been accepted that blossom-visiting insects play an important part in disseminating the fire-blight organism, *Bacillus amylovorus* (Burr.) Trev. Recently, however, the work of Miller (2) and Tullis (5) indicates that this organism can be spread by rain without the aid of insects. An example of this type of dissemination is illustrated by figure 1, in which the upper apple branch was naturally infected with blight in 1929. In the spring of 1930 bacteria escaped from this 1929 infection and were washed by rain to the new growth on lower limbs, where they subsequently infected both leaves and shoot tips. Numerous cases of this type of infection from hold-over cankers were observed in 1930. Such infections indicate the importance of meteoric water in spreading blight bacteria from old bark lesions to new growth nearer the ground. But meteoric water alone could not account for the epiphytotics of blight, especially blossom blight, which have occurred in Tennessee during the past 2 years. Therefore, it must be concluded that blossom-visiting insects are still the most important factor in wide dissemination of fire-blight bacteria.

The work of Rosen (3) gives further evidence that bees are a factor in harboring and spreading the fire-blight organism. The findings of Thomas (4) offer a concrete explanation of the fact that a few pear trees with their blossoms blighted early in the spring serve as centers for infesting bees which spread the bacteria to entire apple orchards when these bloom some weeks later. Even though he succeeded in obtaining virulent blight bacteria from the surface of honeycomb after 55 days, Thomas (4) expresses doubt that such bacteria would have lived over winter in that way. The conclusion is that hold-over cankers are one of the most important known means by which *Bacillus amylovorus* lives from fall to spring.

It is generally agreed that the fire-blight organism may overwinter in both pear and apple cankers. Of the apple varieties, Transcendent crab is one of the most favored hosts for hibernation. Their ability to overwinter the blight bacteria, coupled with their early spring blooming, makes Transcendent-crab trees second only to Kieffer pears as important centers for bee contamination. In the Southern States the Transcendent crab seldom occurs in large numbers, but it is not uncommon to find one or more trees of this variety in home orchards. Since it is prized for jelly making, owners hesitate to destroy their trees to reduce blight of surrounding varieties. In studies on blight control it was therefore important to seek a practical method that did not involve the cutting down of any trees.

FIG. 1. The bare upper limbs were killed by natural blight infection in 1929 and left without treatment. In the spring of 1930 rain carried bacteria from the 1929 infection, causing a number of fresh infections on the new growth of the lower limbs.

Efforts to control fire blight by surgical methods have not been sufficiently successful to convince the average grower that this is a practical method. Failure to get results from surgical methods alone led the writer to turn to the zinc chloride treatment by which Day (1) in California had succeeded in killing blight bacteria in pear cankers. During the past 2 years this treatment has been applied to hundreds of apple and pear cankers of many varieties, but the experiments here reported were confined to the Transcendent-crab variety. Among others treated in 1929 were 3 bearing trees of this variety growing in a test orchard on the University Farm. One of these trees is a standard, the second a semidwarf, and the third a true dwarf, figure 2. These trees had been in bearing for several

years and, regardless of their rootstock, had bloomed, foliated, and set and matured fruit at approximately the same time. During the heavy blight epiphytotic of 1929 all 3 trees became infected. This resulted in the loss of considerable fruit and in the development of numerous blight cankers. Because it was desired to kill the blight bacteria in these cankers regardless of other effects, the strongest solution, 53 per cent, recommended by Day (1) was used in treating all cankers. The solution was painted over all the infected areas and for a distance of 8 to 12 inches beyond external symptoms of blight. The first treatments were marked with dated tags, and the few subsequent infections were similarly treated and labeled.

In the early-season infections it was obvious that the bacteria were spreading rapidly into new tissues prior to treatment, but such advance appeared to cease shortly after the zinc chloride solution was applied. In

FIG. 2. The dwarf Transcendent-crab tree as it appeared during the summer of 1926. It had borne a crop of fruit each year since 1926 but was heavily infected with blight in 1929.

most cases the treatments were made as soon as external symptoms of blight appeared; therefore, a few deep cankers developed. In a few cases, however, the organism entering through short fruit spurs induced deep cankers involving the cambium. The zinc-chloride solution appeared to penetrate these and kill the bacteria the same as in the shallow cankers. By labeling all infections after treatment the writer was sure that these 3 Transcendent-crab trees entered the dormant priod in the fall of 1929 with no untreated cankers.

In order to eliminate insect transmission as far as possible, these 3 trees, while still dormant, were on March 7, 1930, enclosed in wood-frame cages, covered with 16-mesh galvanized-wire screen. The bases of the cages were enclosed with boards set in the ground so as to exclude entrance from that source, and the doors were kept closed except when the writer was entering or leaving a cage.

Although the cages afforded the desired protection from insect infections, yet from the observations that follow it is seen that the results were adverse in that exclusion of insects prevented fruit setting. These Transcendent-crab trees, as usual, bloomed considerably in advance of all other apple varieties; their blossoms were fully open by April 1, 1930. Kieffer pears were in bloom about March 20, 1930, and showed well-developed cases of blossom blight by the time Transcendent-crab trees were in bloom. When the caged crab trees bloomed many blossom-visiting insects were attracted, but very few gained entrance through the 16-mesh screen, and the flowers remained practically free from flying insects throughout the blooming period. The blossoms appeared normal, the pollen was abundant, and the weather bright and favorable for pollination, yet nearly all dropped without setting fruit. During this period no blight developed on any of the 3 caged trees. This indicated that no blight organisms had overwintered in the treated cankers formed on these trees the previous season and that no blight-contaminated insects had reached the blossoms. Neighboring uncaged trees of standard varieties bloomed about April 18, 1930, and blossom and twig blight was abundant on apple trees throughout east Tennessee, but no sypmtoms of infection had developed on any of the other caged trees.

It is now accepted that blossoms are not the only plant parts through which blight bacteria enter the host and that water, as well as insects, plays an important part in bacterial spread. In order to verify these findings under controlled conditions the following experiment was conducted. On May 6, the writer cut from young, bearing apple trees of a number of varieties sufficient blighted fruit spurs and young shoots to fill a 16-quart berry carrier about 7 x 11 x 22 inches inside measurement. During a shower late in the afternoon of the same day the container of blighted twigs was placed

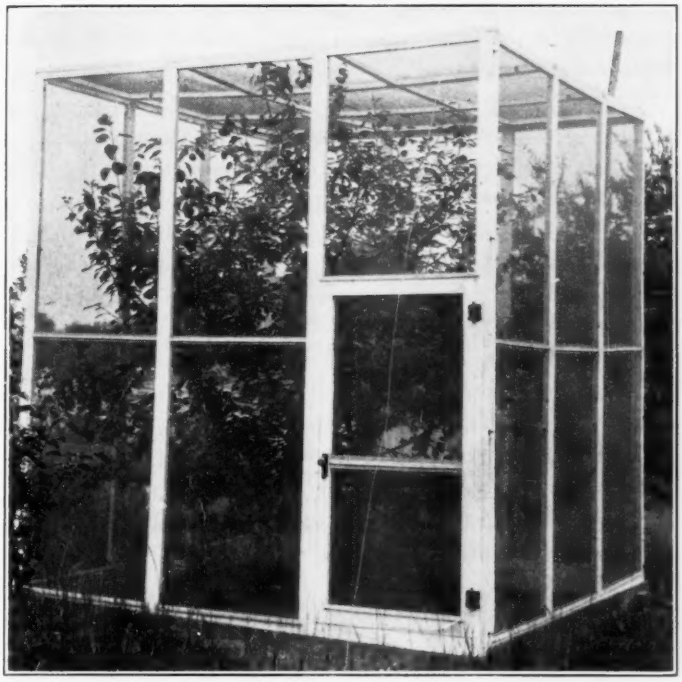

FIG. 3. The standard Transcendent-crab tree which had been in bearing for several years and which was heavily infected with blight in 1929. All infections were treated with 53 per cent zinc chloride solution in 1929, and the tree was enclosed in the cage March 7, 1930, while still dormant. No blight developed on this tree in 1930.

on the screen covering the top of the cage over the semidwarf tree. Cloudy weather with showers occurred on May 7, 8, and 9, followed by a heavy rain on the 10th and rain on the 13th, 16th, and 17th. On June 7, 1930, 3 well-developed cases of twig blight were observed on branches of the caged tree just under the carrier of blighted twigs. No blight had developed on the caged standard tree (Fig. 3), which served as a check; therefore, the blighted twigs on the semidwarf tree were undoubtedly due to bacteria carried to them from the container of blighted fruit spurs and shoots by meteoric water. After removing the carrier from the top of the cage the writer entered, cut the 3 blighted twigs from this tree and disinfected the cut surfaces. The blighted twigs were placed at once in a moist chamber and 2 days later all 3 exhibited drops of bacterial ooze. This ooze was found, by infection experiments, to contain the fire-blight pathogene.

It is generally considered that slowly growing trees are less susceptible to blight, but results obtained with the dwarf Transcendent-crab tree under controlled conditions indicate that this is not always the case. For

on May 12, 1930, blighted blossom spurs and new shoots were cut from a Bartlett-pear tree in the Station plots and placed on the cage covering the dwarf Transcendent-crab tree. No bacterial ooze was observed on these blighted pear shoots at the time they were cut, but the rains of May 13, 16, and 17 must have washed bacteria from them to growing tips of the caged dwarfed tree, for, subsequently, 2 twigs developed typical blight. The caged standard tree developed no symptoms of blight throughout the season; therefore, the blight infections on the dwarf tree must have come from bacteria from the blighted pear twigs on top of the cage. After removal of the blighted tips and disinfection of the cut surfaces no further blight developed on the 2-caged trees.

With blossom-visiting insects excluded from these caged trees, the absence of blight infection during and immediately following the blooming period is proof of the effectiveness of the 53 per cent zinc chloride solution in destroying blight bacteria in hold-over cankers on these apple trees. The fact that 2 of these caged trees were later artificially infected through water-borne bacteria, while the unexposed tree remained healthy, indicates that these trees were equally susceptible to blight in 1930 as when naturally infected in 1929.

In this and other experiments with apples and pears no serious injury to limbs has resulted from treatments of blight cankers with 53 per cent zinc chloride solution. Therefore, these data indicate that much could be done to reduce the primary spread of the blight organism and the spring contamination of bees if the hold-over cankers were given a thorough application of zinc chloride.

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BACTERIAL LEAF SPOT OF VIBURNUM

H. H. Thornberry and H. W. Anderson

Bacterial leaf spot of Viburnum was first observed by the writers on May 25, 1929, on the University of Illinois campus. Further investigations of the Virburnum plantings on the campus revealed infection on certain species only.

SYMPTOMS

Infections are for the most part on the leaves, but young stems are somewhat susceptible. Lesions on the fruits or in the inflorescence were not observed. Leaf lesions are more conspicuous than those on the stems.

The early stage of leaf infection becomes manifest in water-soaked, circular areas. In about 4 days after these areas are visible they develop into irregular, shrunken, brown spots from 2 to 4 mm. in diameter (Fig. 1). The center of aged lesions appears somewhat transparent. Microscopic examination of stained sections of such lesions shows disintegration of the mesophyll cells.

Fig. 1. Natural infection on a leaf of *Virburnum opulus*.

Young lesions of the stem seldom become conspicuous. At first they are water-soaked, elongated, and superficial. Later, they develop into slightly shrunken streaks, limited to the cortical regions of the stem (Fig. 2).

Microscopic examination of affected leaf tissues shows abundance of bacterial ooze from the margin of the lesion, but the shrunken, transparent center of older lesions is free from masses of bacteria. Diseased tissues of stems contain abundance of bacteria which ooze from sections in water.

FIG. 2. Stem lesions in which bacteria live over winter. The lesions appearing as brown shrunken streaks are limited to the cortex.

DISTRIBUTION AND HOST RANGE

The disease has been observed on *Viburnum opulus* L., *V. tomentosum* Thunb., and *V. dentatum* L. on the University of Illinois campus. Other species on the campus, *V. acrifolium*, *V. americanum* Mill., *V. Carlesii* Hemsl., *V. cassinoides* L., *V. lantana* L., *V. lentago* L., *V. molle* Michx., *V. prunifolium* L., *V. Sargentii* Koehne and *V. Sieboldii* Miq., were not infected. Viburnums investigated at other points in the State, including Champaign and Urbana, were free from infection. The disease has not been observed elsewhere and no reference to it has been found in the literature. Since the disease is easily noticeable when in a severe form, it would seem that it could hardly have been overlooked had it been common or widely distributed. A limited number of inspections have been made on wild species of Viburnum and in nurseries without revealing any further distribution.

ISOLATION

The dilution-poured-plate method was used to isolate the causal organism. Small sectors from lesions were placed in a drop of sterile water on a microscopic slide and then examined with low-power magnification. The sectors which showed bacterial ooze were transferred to 1 cc. of sterile water in a sterile Petri dish and allowed to remain 30 seconds or longer, depending on the amount of observed bacterial ooze. By transplanting one loopful of the inoculum to a second Petri dish containing 1 cc. of sterile water, and from the second to the third dish, suitable dilutions were obtained. The plates were poured with melted dextrose agar that had been cooled to 40° C. Typical colonies developed within 4 days when incubated at 25° C. Stock cultures were obtained by transferring from single colonies to dextrose-agar slants.

The organism was reisolated from artificially produced lesions.

INOCULATION

Stomatal entrance was readily demonstrated by atomizing a suspension of the pathogene on young leaves of *Viburnum opulus*. Inoculations thus made on June 2, 1929, during a gentle rain, developed typical lesions within 9 days. Inoculations again made on June 12 resulted in evident infection by June 25. It was observed that rubbing the leaves between the thumb and fingers or scratching them with a sharp needle, while the suspension was atomized, aided entrance (Fig. 3).

OVERWINTERING OF THE PATHOGENE

The pathogene lives through the winter in the tissues of dormant buds and in small cankers on young stems. Primary leaf infection in the spring results for the most part from the inoculum in infected tissues near buds

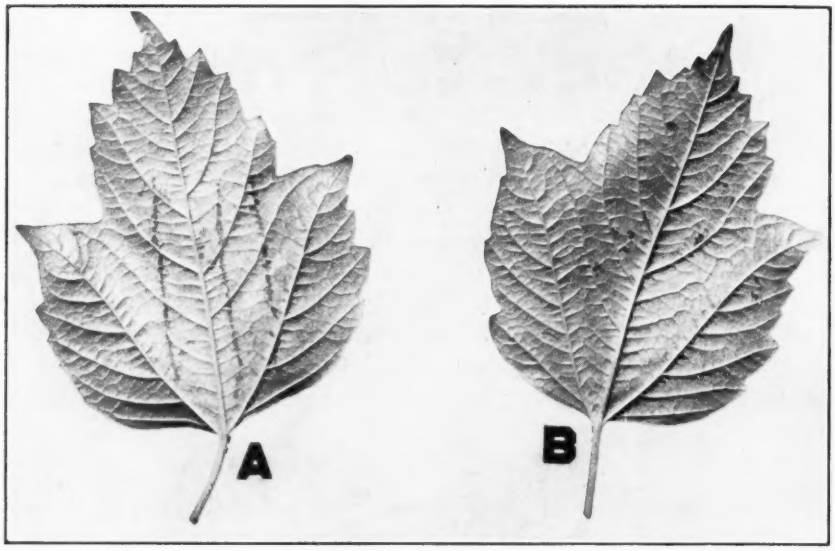

FIG. 3. Artificial infections. A. Inoculation made by atomizing a suspension of the pathogene and by scratching the leaf with a sharp needle. B. Inoculations made by atomizing the suspension without injuring the leaf.

and not from the stem cankers. Bud and stem infections occur during late summer and early fall, before infected leaves drop and while the young stems are succulent. The pathogene probably exists in a semi-active condition in the diseased tissue during the winter months.

Infected buds, which were first observed on April 20, 1930, contained visible lesions in the region of the cortex of the stem adjoining these buds. Stained microtome sections of the bud and adjoining tissue showed bacteria concentrated in definite areas of disintegrated cells. Serial sections showed that infection originated in the stem at the axis of the bud and advanced through the cortex to the basal tissues of the bud. Infection probably occurred during early fall before the infected leaves dropped. Invasion continued for the most part during fall and the organism remained semi-active during the winter months. During spring the pathogene became active and the organism advanced farther into the outer portion of the bud and adjoining tissues. Buds that were severely infected were killed outright.

The temperatures through September and October and April and May were favorable for continuous growth of the organism. Furthermore, meteorological data show that the temperature on several days during each of the winter months was above 12° C., the limit for growth of the organism in culture; thus it was possible for the bacteria to develop during these periods.

Primary leaf infections were evident April 28, 1930, 1 week after buds opened. Since the incubation period is 7 to 9 days, natural infection must have occurred when the buds expanded. The severity of local infections on leaves developed from certain buds (Fig. 4), and the absence of general infection indicates that the infected buds were the source of inoculum. Local infections in all cases were associated with stem lesions near the original position of the bud.

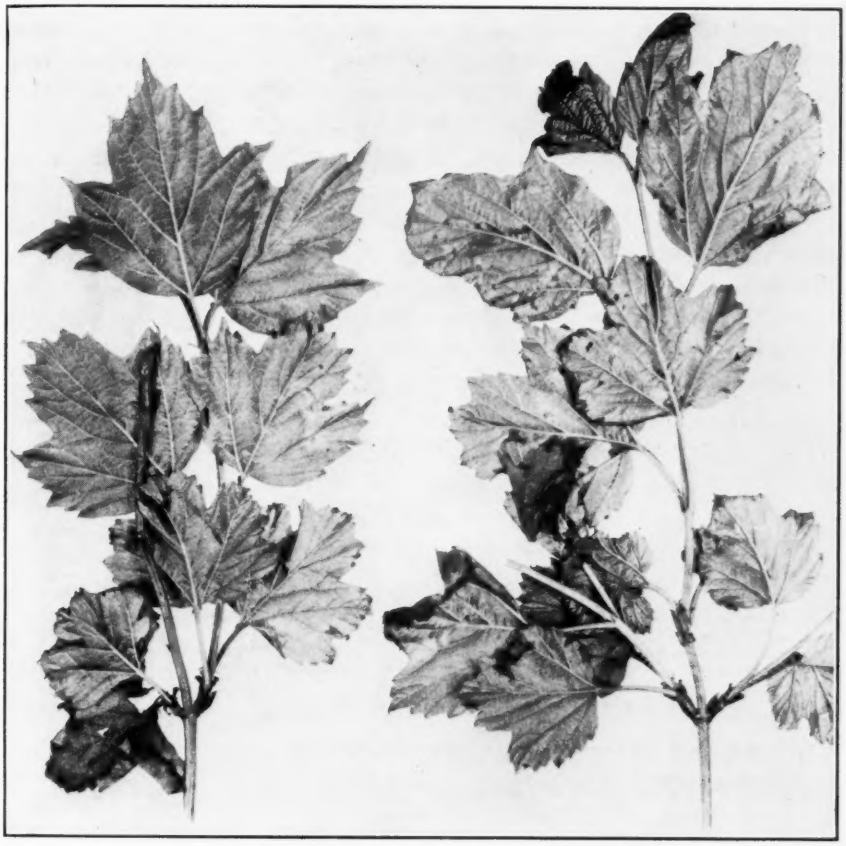

FIG. 4. Primary spring infections, traced to lesions under the bark of the terminal portion of the stem.

The pathogene remained viable through the winter months in cankers, but in no case were primary infections traceable to them. This inoculum, as well as that from buds killed from severe infection and from overwintering bacteria in the infested leaf débris in the soil, no doubt is capable of producing infection, but not until the leaves are evolved. By this time primary infections from buds are well established.

DESCRIPTION

Phytomonas viburni n. sp. is a short rod 1 to 2 microns long by 0.5 to 1 micron wide, motile by means of 2 or 4 polar flagella, occurring singly, in pairs, and in short chains, but mostly in pairs. It is capsulate but forms no spores; is gram-positive and not acid-fast; forms round, entire, pale dull gray colonies on dextrose beef-extract agar; clouds bouillon and forms a pellicle; has no diastatic action; does not liquefy gelatin; does not ferment xylose, rhamnose, glucose, mannose, galactose, fructose, lactose, maltose, sucrose, rhaminose, raffinose, dextrin, inulin, glycerol, mannitol, sorbitol, dulcitol, nor salicin; does not reduce nitrates; produces an alkaline reaction and causes neither coagulation nor peptonization in litmus milk without reduction of litmus; produces neither hydrogen sulphide nor indol; is aërobic; optimum, minimum, and maximum pH for growth are 8.5, 4.8, and 10.4, respectively; optimum, minimum, and maximum temperatures for growth are 25°, 12°, and 35° C., respectively; causes brown irregular spots on leaves and elongate lesions on petioles, young shoots, and twigs of species of arrowwood, *Viburnum opulus*, *V. tomentosum*, and *V. dentatum*.

A culture of *Phytomonas viburni* has been sent to the American Type Culture Collection, John McCormick Institute of Infectious Diseases, Chicago, Illinois.

SUMMARY

1. Bacterial spot of Viburnum was discovered May 25, 1929, on the University of Illinois campus.

2. The disease is apparently of local distribution.

3. The pathogene lives over winter in cankers, in infected buds, and possibly in infested leaf débris in the soil. Primary infection in all cases observed developed from infected buds.

4. Artificial inoculations produced typical lesions and the organism was reisolated in pure culture.

5. A description is given of *Phytomonas viburni*, the causal organism.

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DIDYMOSPHAERIA OREGONENSIS, A NEW CANKER ORGANISM ON ALDER[1]

L. N. Goodding

INTRODUCTION

Field studies on white-pine blister rust in the Pacific Northwest have led to some observations on other fungi affecting forest trees. In 1929 the writer noticed a peculiar trunk canker on alder (*Alnus rubra* Bongard). At first glance it appeared to be the result of insect work or injury due to sapsuckers. Closer examination, however, proved it to be a well-defined canker having a fungus constantly associated with it.

A study of the fungus has shown that it is an undescribed Didymosphaeria. L. E. Wehmeyer, of the University of Michigan, in a letter dated April 26, 1930, suggested that the most closely related Didymosphaeria previously described is *D. nana* var. *brachyspora* Sacc.[2] This occurs on the leaves of an Alaskan alder. Since, however, the species here discussed is found only on the trunks and limbs of the alder and is obviously different from one other close affinity in the genus, the writer proposes the name **Didymosphaeria oregonensis** n. sp.

DISTRIBUTION

This fungus is general throughout western Oregon. Two specimens were collected in northeastern Oregon, one in western Washington, and two in Idaho. It probably is common throughout the Northwest. This fungus has been found on three species of alder; namely, *Alnus rubra, A. tenuifolia* Nutt., and *A. sinuata* (Regel) Rydb. It is worth noting that abundant *Betula glandulosa* Michx. associated with alder sustaining a heavy infection of *Didymosphaeria oregonensis* at Horse Thief Meadows, Hood River County, Oregon, showed no signs of infection.

ECONOMIC IMPORTANCE

The constant association of this organism with the marked banding effect and at times with the pronounced swollen cankers on the boles and branches of alder indicates that it is an active parasite. There seems to be some indication from observations in the Mud Creek region in the Mt. Hood National Forest that it becomes sufficiently active at times to kill the affected branches. This organism, however, has not been shown to be the cause of this killing. In many localities a mere band of roughened bark is

[1] This has previously been mentioned in a brief abstract in Phytopath. **20**: 854. 1930.

[2] Sacc. Syll. Fung. **17**: 679. 1905. (See also Harriman Alaska Expedition **5**: 30. 1900.)

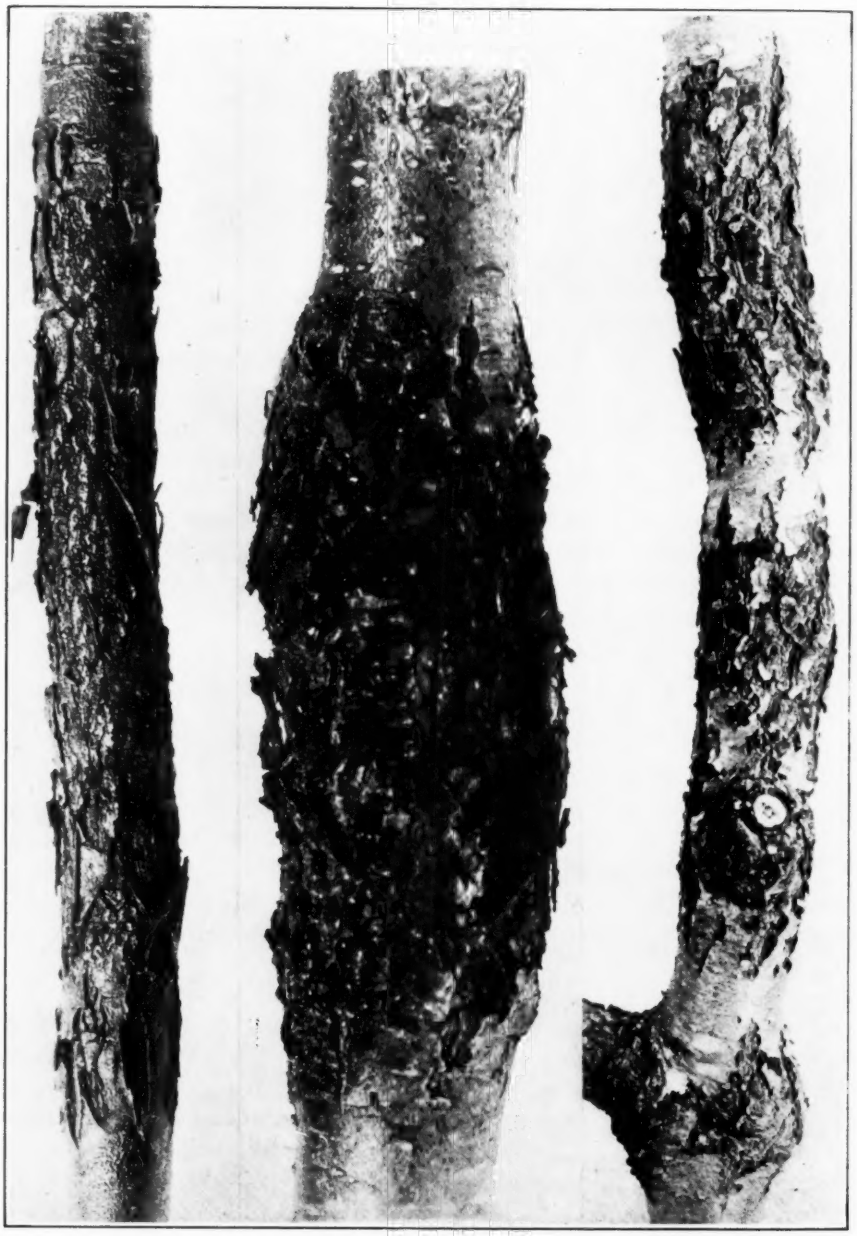

FIG. 1. Photograph of cankers on *Alnus rubra* caused by *Didymosphaeria oregonensis* Goodding. Natural size.

produced, but in others, or on certain trees, each canker forms a pronounced swelling. In no place where it has been observed can it be considered a serious pest, though in some localities young trees are deformed and stunted by very numerous cankers.

DESCRIPTION OF CANKER AND FUNGUS

Cankers with which the fungus is associated form bands around the trunks and limbs of the alder (Fig. 1). These bands vary from ½ inch to 2 feet or more in length. The organism apparently confines its activity to young limbs and trunks, ceasing to grow after the bark has become hard and thick. The scars, however, are often evident on the trunks of mature trees. While working on the tender bark the organism produces a marked zoning which readily reveals the age of simple cankers. A longitudinal section of a canker where swelling has taken place shows increased thickness of the annual layers following infection. This apparently is due to the release of tension where the fungus breaks the outer bark. Perithecia are produced only on the portion of the bark recently invaded. In the very early spring a slight discoloration of the bark beyond the band bearing the perithecia of the last season can be clearly discerned. By early May this reveals the growing perithecia by a pronounced pimpling. By midsummer these are producing mature ascospores. Perithecia are numerous, single or occasionally somewhat caespitose, those of the current season confined to a zone from a few millimeters to several centimeters wide. If this consti-

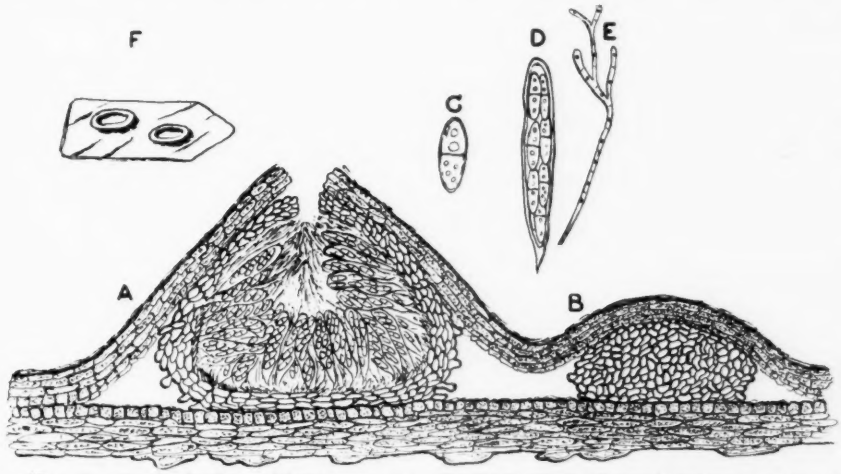

FIG. 2. A. A single perithecium. ×30. B. Wall of single perithecium. ×30. (A and B show the outer cortical layer raised by the perithecia.) C. A single ascospore. ×400. D. An ascus with spores. ×275. E. A paraphysis. ×275. F. A scrap of inner bark showing collapsed perithecia. ×5.

tutes the initial growth it starts as a narrow lens-shape area but soon forms a complete single band about the trunk or branch. As the perithecia reach maturity the outer cork layer of the bark is broken loose and raised. Finally, the outer layer of the bark exfoliates, leaving a roughened and ragged surface. The perithecia usually remain attached to the bark on the trunk or branch but occasionally peel off with the outer cork layer. After the contents of a perithecium are exuded, the upper portion collapses and the base forms a minute cup.

The technical diagnosis follows:

Didymosphaeria oregonensis n. sp.

Perithecia (Fig. 2) about 1 mm. across, globose, dark brown or black, opening by a very minute pore which does not protrude beyond the surface of the bark; the cavity is filled completely with the asci and filiform, branched, septate paraphyses; *asci* numerous, produced from the sides and bottom of the perithecia, cylindrical to clavate or quite irregular, 75–90 μ long, opening by a wide pore at the tip which does not turn blue with iodine, contain 8 spores; *ascospores* "kildare green" (Ridgway), rounded at both ends, 1-septate, slightly if at all constricted at the septum, 18–21 × 7–9 μ.

This organism apparently is related to *Didymosphaeria Wallrothii* (Hipp.) Sacc. & Trott.[3] from which it differs in having larger spores, less conspicuous perithecia, and nonprotruding ostioles. *D. Wallrothii* occurs on birch bark and has not been reported from North America. As suggested by Wehmeyer, *D. oregonensis* may also be related to *D. nana* var. *brachyspora* Sacc. In *D. oregonensis*, however, the spores are relatively much broader than those in *D. nana* var. *brachyspora*. This and the fact that *D. oregonensis* has never been reported as occurring on the leaves indicate that it is a distinct species.

SPECIMENS EXAMINED

On *Alnus rubra* Bongard

Oregon: Clackamas County, Still Creek, *L. N. Goodding*, May 30, 1929, 4961,[4] July 10, 1929, 4963, May 23, 1930, 5533, *L. N. Goodding* and *A. L. Hinckley*, July 4, 1930, 5534 and 5535; Mud Creek, *L. N. Goodding* and *G. D. Darker*, July 12, 1929, 4964; Yokum Falls, *L. N. Goodding* and *G. D. Darker*, July 15, 1929, 4965, *TYPE;* Crater Lake, *L. N. Goodding* and *M. C. Riley*, August 4, 1929, 4966; Colla-

[3] Sacc. Syll. Fung. 1: 715. 1882. 22: 174. 1913.

[4] All numbers refer to the Oregon State College Herbarium.

wash River, *L. N. Goodding* and *M. C. Riley*, August 16, 1929, 4968; Camp Creek, *L. N. Goodding* and *M. C. Riley*, October 13, 1929, 4967; Zig Zag River, *L. N. Goodding* and *M. C. Riley*, October 15, 1929, 4972; Rhododendron, *L. N. Goodding*, March 5, 1930, 4977, April 18, 1930, 5531, *L. N. Goodding* and *E. W. Lyle*, October 2, 1930, 5532; Columbia County, Vernonia, *L. N. Goodding*, March 19, 1930, 4978; Douglas County, Gunter Road, *L. N. Goodding*, November 23, 1929, 4975; Florence, *L. N. Goodding*, November 25, 1929, 4976; Canyonville, *L. N. Goodding*, February 22, 1930, 5527; Hood River County, Eagle Creek, *H. N. Putnam* and *L. N. Goodding*, October 18, 1929, 4973, *E. L. Joy* and *L. N. Goodding*, May 13, 1930, 4981; Hood River, *L. N. Goodding*, June 4, 1930, 5538; Horsethief Meadows, *L. N. Goodding* and *A. L. Hinckley*, June 5, 1930, 5539; Jackson County, Woodruff Meadow, *F. P. Sipe* and *L. N. Goodding*, August 6, 1930, 5541; Lane County, Triangle Lake, *F. P. Sipe*, September 13, 1930, 5528; Marion County, Breitenbush River, *M. C. Riley*, August 20, 1929, 4969; Polk County, Rickreall Creek, *L. N. Goodding*, June 8, 1929, 4962; Salmon River, *L. N. Goodding* and *M. C. Riley*, September 19, 1929, 4970; Black Rock, *L. N. Goodding*, April 23, 1930, 4979; Tillamook County, Boulder Creek, *L. N. Goodding*, October 25, 1929, 4974; Wasco County, Clear Lake Creek, *L. N. Goodding* and *M. C. Riley*, October 12, 1929, 4971.

Washington: Pierce County, Kantz Creek, *L. N. Goodding* and *M. C. Riley*, May 4, 1930, 4980.

On *Alnus tenuifolia* Nutt.

Oregon: Clackamas County, Summit Ranger Station, *L. N. Goodding*, May 23, 1930, 5530; Deschutes County, Tumalo Creek, *L. N. Goodding*, October 7, 1930, 5529; Umatilla County, Pearson Ranger Station, *E. W. Lyle* and *A. L. Hinckley*, July 4, 1930, 5542; Union County, Jarbeau Creek, *E. W. Lyle* and *A. L. Hinckley*, July 21, 1930, 5540.

Idaho: Clearwater County, Three Bears Creek, *C. C. Strong*, July 9, 1930, 5543; Rhodes Creek, *E. L. Joy*, September 5, 1930, 5544.

On *Alnus sinuata* (Regel) Rydb.

Oregon: Clackamas County, Mud Creek Trail, *G. D. Darker* and *L. N. Goodding*, July 11, 1929, 4982; Mud Creek, *L. N. Goodding* and *A. L. Hinckley*, June 4, 1930, 5536; Wapinitia, *L. N. Goodding*, June 4, 1930, 5537.

SUMMARY

This paper describes as new a species of Didymosphaeria found associated with a canker on three species of living alder in the Pacific Northwest. The name **Didymosphaeria oregonensis** Goodding is proposed. The known distribution of the new species is given as Oregon, Washington, and Idaho. There is also included a brief consideration of its relationship and economic importance.

OFFICE OF BLISTER RUST CONTROL,
UNITED STATES DEPARTMENT OF AGRICULTURE,
STATION AT OREGON STATE AGRICULTURAL COLLEGE,
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THE KELM MOUNTAIN BLISTER-RUST INFESTATION

WALTER H. SNELL[1]

During the past 15 years of intensive search for white-pine blister-rust infestations, momentary enthusiasm has given rise, in a large number of cases, to estimates of practically 100 per cent infection on various lots. In nearly all such infestations, the actual percentage of trees diseased has been found to be below 75 per cent and usually nearer to 50 per cent. There is at least one area (the only one known to the writer), however, in connection with which the original estimate was not extravagant. This Kelm Mountain lot is an outstanding example of blister-rust damage. Because of its inaccessibility, this plot is probably the least known to pathologists and foresters of all blister-rust plots; few have seen it and not many more have heard of it.

This area was discovered during a deer hunt by A. E. Fivaz and E. G. Woodward, of the Division of Blister Rust Control. Inability to find a single uninfected tree in the course of two casual inspections made the area of unusual interest from the point of view of blister-rust damage, and the writer established an experiment plot there in 1923. The plot is at the foot of Kelm Mountain, between the Chestertown and Horicon roads out of Warrensburg, in Warren County, New York. It can be reached only after a walk, mostly uphill, of about a mile and a half from either highway. The elevation of the lot is 1,200 feet. The infested area is in a sort of pocket in the mountains protected on one side by the steep wall of Kelm Mountain and near a small unnamed pond, not far from Kelm Pond. The lot is surrounded by mature pine and hardwood. In general, moisture conditions favorable for the propagation and development of fungous diseases seem to prevail. During the summer the undergrowth usually remains wet until nearly noon, fog is very common in the nights and early morning, the woods are dark, and the forest floor supports an unusually abundant mushroom growth.

The permanent plot consists of 2 acres, selected from an extended stand of white pine of uniform type. This plot, in its original condition, had 550 trees to the acre, divided quite sharply into 2 groups—one of open growth of 400 pines an acre and the other of about 3,000 trees an acre. In the dense part of the plot it appeared that, under normal conditions, an excellent stand would have been produced, since the competition had resulted in the development of well-spaced dominant trees with an undergrowth of badly suppressed trees that soon would have been eliminated by shading. The open part likewise promised a good stand. It is true that there were

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too few pines for this purpose, but the stand was so interspersed with hardwoods of the proper size as to provide the pines with the stimulus for height growth and the shading for clean boles.

At the time of the first study, in 1923, these pines were mostly 10 to 14 years of age. Those that now survive are therefore between 17 and 21 years old.

The open spaces in the stand are quite heavily covered with blackberry brambles. In 1923 there were 174 bushes of *Ribes rotundifolium* Michx., with a total leaf-bearing stem of nearly 9,000 feet in the open part of the plot. No bushes were found in the dense part of the plot nor in the surrounding mature-pine and hardwood growth. In 1923, 25 per cent of the leaf-bearing stems were dead and 50 per cent were dead in 1926. At the present time, the few surviving bushes are in very poor condition. They are, however, numerous enough to provide infective material for the new pine seedlings as they appear.

As far as can be determined, this land was cleared sometime prior to 1907, when the owner sold the property and moved away. This fact is corroborated by the age of the trees now on the plot.

In all, during the past 8 summers, 1,110 trees have been found on this plot. Of these, 96.5 per cent have been found infected at one time or another. There are at present only 1,006, or 90.6 per cent, with living cankers. The cankers on 64 trees (6 per cent) are now dead because of shading. About 9,000 cankers, in all, have been found on the plot, an average of about 9 per tree. One tree bore 298 cankers, and 7 trees had between 100 and 200. The cankers on the trees in 1924 were distributed as follows: 0.4 per cent on 1916 wood, 3 per cent on 1917 wood, 16 per cent on 1918 wood, 74 per cent on 1919 wood, 5.6 per cent on 1920 wood, and 1 per cent on 1921 wood.

There was not a dead tree on the lot in 1920. In 1923, 9 per cent were dead; in 1924, 14 per cent; in 1926, 30 per cent; in 1927, 42 per cent; in 1929, 60 per cent; and, in 1930, 69 per cent. There are still left on the plot 18 per cent of the entire number that will die in a few years, making an imminent mortality of 87 per cent of the entire stand.

There will be left on the lot 147 noninfected trees. Of these, however, 40 will not survive because of shading or other factors. This will leave only 107 living trees on the 2 acres. Most of these are severely suppressed.

There is at the present time no reproduction to make a new stand, nor have any seedlings matured since 1910. All of the young trees that presumably were seeded in between 1910 and 1920 were killed. We are certain that there must have been some reproduction in that decade, because there is reproduction on other lots not infested with the disease. The years 1911, 1913, 1915, 1917, and 1919 were good seed years in the Adirondacks.

There has been seeding in years since 1920, but most of these young trees are at present infected and are doomed to die. This plot is a good example of the 2-storey condition explained in former publications[2,3] except that, because of the continued severity of the disease, the lower storey (trees that seeded in since 1920) is sparser here than in most places.

The original stand of 2 acres, one portion of pure pine and the other mixed with hardwoods, was such that, undisturbed by disease, it probably would have produced a minimum of 60,000 and probably nearer 70,000 board feet of high quality lumber. The rust will have left only 147 trees noninfected and, of these, shading will remove all but 107. The 107 trees that will survive rust and shade (presupposing that no more of these are attacked by the rust in future years) will be worth almost nothing and the damage because of blister rust will therefore be practically complete.

To one who has followed the conditions on this plot since the earliest studies, the change within the past few years is very striking. Up to 1926, to casual observation, the plot looked like a thriving young pine stand, practically pure pine in one part and mixed with hardwoods in the other. Few dead or dying trees were visible when the lot was viewed from the open. Whereas, at that time, the vigorous pines were visible even in the hardwoods, showing through and crowding above them, there is now little pine foliage to be seen. The single pines in the hardwoods are dead and dying, and, in the thicker portions, most of the pines are already dead. At the present time, it is most strikingly evident that the lot is no longer a pine stand but that it has, within the space of a few years, become a hardwood stand.

Another interesting feature of this stand is the 147 pines that have withstood the blister-rust attacks of the 1910–1920 decade, especially the severe wave of the latter part. These trees, found free from disease in 1923, are still intact. That they are constantly exposed to attack is shown by the diseased condition of all the young trees that have come up since 1919. These trees apparently are immune from the blister-rust fungus. It is going to be of considerable interest to find out how offspring from the seeds of the 107 staunch survivors react to *Cronartium ribicola* Fischer. It is hoped that such tests can be made.

ALBANY, NEW YORK.

[2] Snell, Walter H. Blister rust in the Adirondacks. Jour. For. **26**: 472–486. 1928.

[3] Snell, Walter H. Some observations on the white pine blister rust in New York. Phytopath. **19**: 269–283. 1929.

A WITCHES' BROOM OF OCEAN SPRAY (HOLODISCUS DISCOLOR)[1, 2]

S. M. ZELLER

In the spring of 1925 a resident of Corvallis, Oregon, observed in his garden a "freak" plant of ocean spray, *Holodiscus discolor* Max. He had secured the plant from an open woodland in Marion County, Oregon. Ocean spray is a beautiful rosaceous shrub, native to the Pacific Northwest. We have chosen to call the abnormal condition of this ornamental shrub a "witches' broom." Many diseased plants have been observed in the foothills of the western slope of the Cascade Mountains in Oregon from Wasco, Hood River, and Multnomah counties in the north through Clackamas, Marion, and Linn counties to the south. One specimen was brought in by L. N. Goodding from west of Olympia, Washington.

When this disease first makes its appearance on a plant the new lateral branches from an old stem are very slender and wirelike, with rather short internodes and small leaves. As a rule, there are 2 or 3 of these slender laterals from each node, while in healthy plants the laterals are more stalky and but 1 from each node. In the second or third year there is considerable multiplication of the laterals from each node on stems 2 or more years of age, and these laterals are much branched in contrast to the laterals of healthy plants.

New canes, which arise from or near the crown after the plants become affected, are short and give a stiff appearance, as illustrated (Fig. 1). In these canes the internodes are short; the main stems have little tendency to branch, so there are usually no blossom clusters. There are several buds at each node and these produce very short spindly laterals.

The leaves of affected plants are very small and crowded, giving the canes a very leafy appearance. Where they are not shaded they turn a bronzy red early in the summer. This general reddish tone may appear early in June when the spring is cool. This color stands out in contrast to the bright green of neighboring healthy plants.

Plants showing symptoms of the disease on 1 cane only were marked to determine subsequent development. As a rule, all of the aboveground portions of the plant showed symptoms the following year. All new canes from below or just above the ground in such cases showed advance symptoms of the disease.

In the early spring of 1927, buds from diseased plants were grafted into large healthy stems, 1 year old. In 6 out of 15 trials, the buds grew and

[1] Published with the approval of the Director as Technical Paper No. 136 of the Oregon Agricultural Experiment Station.

[2] An abstract of this paper appeared in Phytopath. 29: 851. 1930.

FIG. 1. Two branches of ocean spray affected with witches' broom. The middle upright branch is from a healthy plant.

in all 6 cases the disease was transmitted. The grafted node in such cases is the first to show symptoms. Then, the new growth appearing on the stock above the grafted bud shows a spindliness, but, during the remainder of the season, no symptoms are evidenced in other canes of the plant. In the second season (1928), however, in all 6 cases the whole plant showed evidence of the disease and the new canes were dwarfed with typical, advanced symptoms.

In an effort to discover the possibilities of insect transmission small laterals from diseased plants with aphis, *Aphis spiraecae* Schout., on the young leaves were placed in considerable quantity on 10 healthy plants on May 10, 1927. No symptoms of the disease made an appearance that season. In the spring of 1928, however, on 4 of these plants all of the new canes from near the ground and all of the lateral branches of any size showed rather advanced symptoms of the disease. On May 16, 1928,

a similar transfer of aphids to 12 healthy ocean-spray plants was repeated and, at the same time, aphids from diseased plants were transferred to 4 plants of *Spiraea thunbergii* Sieb., 2 plants of *S. Vanhoutei* Zabel, 1 plant of *S. prunifolia* Sieb and Zucc., 7 plants of *S. Douglasii* Hook., and 3 plants of *Physocarpus capitatus* (Pursh.) Ktze. In the summer of the next year, 1929, 9 of the 12 plants of ocean spray showed symptoms of witches' broom, but none of these other closely related rosaceous plants, to which aphis had been transferred, showed any indication of the disease. No disease resulted where aphids were transferred from healthy plants to 14 healthy plants. This work was done in the open field where adequate controlled conditions were not available, but the results appear sufficiently consistent to warrant notice. The possibility remains that there may be other means of transmission than the two suggested.

The tissues and buds of affected plants have been examined for a possible causal fungus or insect, such as mites or nematodes, but, so far, none has been found. The symptoms and performance of the abnormal condition thus far observed suggest that it might belong to the ever-increasing list of so-called virus diseases.

SUMMARY

A description is given of a disease which causes broominess and dwarfing of *Holodiscus discolor* (ocean spray), an attractive native shrub of western North America. The disease, called "witches' broom," has been found in the foothills of the western slope of the Cascade Mountains from Linn to Wasco counties in Oregon and in Thurston County, Washington. Budding of diseased nodes into healthy stems and the transfer of *Aphis spiraeae* from diseased to healthy plants have both apparently induced the disease. The symptoms and performance of the disorder suggest that it may be a virus disease.

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